

Final Technical Report
SERDP Project CP 1157

**Speciation, Fluxes, and Cycling of Dissolved
Copper and Zinc in Estuaries: The Roles of
Sediment Exchange and Photochemical Effects**

Submitted 12 July 2005

Report Documentation Page			Form Approved OMB No. 0704-0188		
Public reporting burden for the collection of information is estimated to average 1 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing the collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden, to Washington Headquarters Services, Directorate for Information Operations and Reports, 1215 Jefferson Davis Highway, Suite 1204, Arlington VA 22202-4302. Respondents should be aware that notwithstanding any other provision of law, no person shall be subject to a penalty for failing to comply with a collection of information if it does not display a currently valid OMB control number.					
1. REPORT DATE 12 JUL 2005		2. REPORT TYPE Final		3. DATES COVERED -	
4. TITLE AND SUBTITLE Speciation, Fluxes, and Cycling of Dissolved Copper and Zinc in Estuaries: The Roles of Sediment Exchange and Photochemical Effects		5a. CONTRACT NUMBER			
		5b. GRANT NUMBER			
		5c. PROGRAM ELEMENT NUMBER			
6. AUTHOR(S) Dr. Stephen A Skrabal (Univ NC) Dr. Ignacio Rivera-Duarte (SPAWAR)		5d. PROJECT NUMBER CP 1157			
		5e. TASK NUMBER			
		5f. WORK UNIT NUMBER			
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) University of North Carolina Department of Chemistry 601 South College Road Wilmington, NC 28403 SPAWAR Systems Center Code 2375 53475 Strothe Road San Diego, CA 92152-6325		8. PERFORMING ORGANIZATION REPORT NUMBER			
9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES) SERDP Program Office 901 N Stuart Street, Suite 303 Arlington, VA 22003		10. SPONSOR/MONITOR'S ACRONYM(S)			
		11. SPONSOR/MONITOR'S REPORT NUMBER(S)			
12. DISTRIBUTION/AVAILABILITY STATEMENT Approved for public release, distribution unlimited					
13. SUPPLEMENTARY NOTES The original document contains color images.					
14. ABSTRACT Copper (Cu) and zinc (Zn) have numerous sources in naval shipping, berthing, and maintenance facilities. Concentrations of these metals in estuaries and harbors can reach levels which are potentially toxic to biota. Hence, discharges and concentrations of these metals are strictly regulated by environmental quality agencies. Because of pervasive problems associated with discharges and remediation of Cu and Zn in estuaries and harbors, it is essential that regulated agencies and regulators alike understand the factors that influence the availability and cycling behavior of these metals in environments relevant to DoD operations.					
15. SUBJECT TERMS Estuaries, copper, zinc					
16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT UU	18. NUMBER OF PAGES 104	19a. NAME OF RESPONSIBLE PERSON
a. REPORT unclassified	b. ABSTRACT unclassified	c. THIS PAGE unclassified			

This page intentionally left blank

Table of Contents

List of Figures	iv
List of Tables	vi
List of Abbreviations and Acronyms	vii
Acknowledgements	ix
Executive Summary	1
1. Objectives	5
2. Background	6
2.1. Overview	6
2.2. Cu and Zn biogeochemistry	7
2.3. The role of sediments in Cu and Zn speciation	8
2.4. Cycling and transformations of Cu and Zn and their complexing ligands	9
3. Materials and Methods	13
3.1. Sampling	13
3.2. Benthic flux experiments	15
3.3. Photochemical experiments	15
3.4. Metal speciation	16
3.5. Total dissolved Cu and Zn	17
3.6. Dissolved organic carbon	18
3.7. Acid-leachable Cu in sediments	18
3.8. Sediment organic carbon and water loss	19
3.9. Dissolved Cu(I) determination	19
3.10 Contamination control	19
4. Results and Accomplishments	20
4.1. Overview	20
4.2. Total dissolved Cu and Cu speciation in the Cape Fear estuary	20
4.3. Benthic fluxes of TDCu and strong Cu-complexing ligands	26
4.4. Total dissolved Zn and Zn speciation in the Cape Fear estuary	31
4.5. Benthic fluxes of TDZn and $\Sigma L_{i,Zn}$	39
4.6. Comparison of Zn and Cu Speciation and Fluxes	43
4.7. Characterization of Zn-complexing ligands in the Cape Fear estuary	44
4.8. Photochemical irradiation experiments: Water column	46
4.8.1. <i>Impact of Cu on CDOM photodegradation</i>	46
4.8.2. <i>Photochemical production of Cu(I)</i>	49
4.9. Photochemical degradation of strong Cu-complexing ligands	51
4.10. Photochemical sediment resuspension experiments	53
4.11. Cu redox behavior	60
4.12. Collaborative studies with SERDP projects CP-1156 and CP-1158	64
4.12.1. <i>Interlaboratory comparison of total dissolved metals and metal speciation</i>	64
4.12.2. <i>Collaborative sampling and ultrafiltration studies of the Cape Fear estuary</i>	70
4.12.3. <i>Benthic flux studies in San Diego Bay</i>	72
4.13. Response to action item	74
4.13.1. <i>Sunlight effects and implications</i>	74
4.13.2. <i>Dredging effects and experimental design</i>	75
5. Conclusions	76

5.1. Interlaboratory comparisons of total dissolved Cu and Zn and Cu and Zn speciation.....	76
5.2. Total dissolved Cu and Zn in the Cape Fear estuary	76
5.3. Dissolved Cu speciation in the Cape Fear estuary and implications for other estuarine systems.....	76
5.4. Dissolved Zn speciation in the Cape Fear estuary and implications for other estuarine systems.....	77
5.5. Benthic fluxes of Cu and Zn and Cu- and Zn-complexing ligands in the Cape Fear estuary and implications for other estuarine systems.....	77
5.6. The role of photochemical processes in affecting the speciation and cycling of Cu in the Cape Fear estuary and implications for other estuarine systems	78
5.7. The effects of dredging on Cu and Zn cycling in the Cape Fear estuary	79
Literature Cited	81
Appendix A: Supporting Data	90
Appendix B: Technical Publications	91
B.1. Articles or papers published in peer-reviewed journals.....	91
B.2. Technical reports.....	91
B.3. Conference/symposium proceedings and/or papers scientifically recognized and referenced (other than abstracts).....	91
B.4. Published technical abstracts	92
B.5. Published text books or book chapter	93
Appendix C: Other Technical Material.....	94
C.1. Scientific/technical honors received	94

List of Figures

Figure 1. Map of sampling sites in Cape Fear estuary.	14
Figure 2. Distribution of DOC with respect to salinity for completed Cape Fear estuarine transects from February 1999 to September 2000.	23
Figure 3. Relationship between $\Sigma L_{i,Cu}$ and DOC concentrations for the Cape Fear estuary (February 1999 to September 2000).	24
Figure 4. Relationship between $\Sigma L_{i,Cu}$ and DOC concentrations in ultrafiltered size fractions of Cape Fear estuarine water (April 2002).	24
Figure 5. Comparison of $\Sigma L_{i,Cu}$ vs. projected DOC relationship for prepared Cape Fear humic solutions and $\Sigma L_{i,Cu}$ vs. DOC relationship measured for Cape Fear estuarine transect data.	25
Figure 6. Relationship between TDZn and Zn-complexing ligands from bottom waters at (a) station 1 and (b) station 2	33
Figure 7. Relationship between strong Zn-complexing ligands and DOC for all sampling times at stations 1 and 2 in the CFE.	35
Figure 8. Relationship between DOC concentrations and river flow, showing a statistically significant correlation.....	36
Figure 9. Relationship between TDZn and Zn-complexing ligands and river flow from bottom waters at stations 1 and 2 in the CFE during all sampling times.....	36
Figure 10. CLE-CSV titration curves in the (a) presence and (b) absence of C_{18} -extracted humic substances in UV-irradiated seawater of salinity ~ 10	38
Figure 11. Typical titration curves for Zn speciation in the (a) presence and (b) absence of Zn-complexing ligands.	45
Figure 12. Ruzic-van den Berg linearization indicating the presence of one class of Zn-complexing ligands. March 2002, sta. 2, timepoint T0.....	45
Figure 13. Change in absorbance between light-treated and dark control samples as a function of wavelength for a 0.22 μm -filtered CFE sample collected October 2000 at 1.5 m depth.	47
Figure 14. Increasing concentrations of Cu(II) added to CFE water increase the degree of protection again photobleaching following exposure to simulated sunlight.	47
Figure 15. Linear regression of % photoprotection versus added Cu for data in Figure 14.	48
Figure 16. Concentrations of strong Cu-complexing ligands and Cu(I) in CFE samples	50
Figure 17. Photolytic degradation of strong Cu-complexing ligands in a CFE sample in solar simulator.	52
Figure 18. Change (Δ) from initial values to after 9 h of Cu (nM per g dry sediment) for triplicate light-exposed and dark controls of 0.2 μm -filtered CFE water with added sediment.	57
Figure 19. Percent organic carbon from triplicate dry sediment samples vs. Cu in the CFE	59
Figure 20. Change (Δ) in Cu(I) concentration (nM) for dark controls and irradiated samples after 9 h compared to initial values, for 0.2 μm -filtered CFE water in triplicate samples from two sites.....	61
Figure 21. Change (Δ) in Cu(I) concentration (nM) for dark controls and irradiated samples after 9 h compared to initial values, for unfiltered CFE water in triplicate samples from two sites.	62

Figure 22. Change (Δ) in Cu(I) concentration (nM/g dry sediment) for dark controls and irradiated samples after 9 hours compared to initial values, for 0.2 μ m-filtered CFE water triplicate samples with added sediments (2 g wet sediment per L) from two sites.....	63
Figure 23. Plot of detected Cu-complexing ligand concentrations versus $\log \alpha_{\text{CuAL}}$. The latter quantity expresses the competition level exerted in the speciation determination experiment, with a larger $\log \alpha_{\text{CuAL}}$ expressing the ability of the experiment to detect stronger classes of ligands.	68

List of Tables

Table 1. Physical parameters, DOC, TDCu, and Cu speciation data for Cape Fear estuarine transects.....	22
Table 2. Individual core and mean flux measurements for core incubation experiment #1 conducted during April 2001.....	27
Table 3. Individual core and mean flux measurements for core incubation experiment #2 conducted during June 2001.....	28
Table 4. Individual core and mean flux measurements for core incubation experiment #3 conducted during October 2001.	29
Table 5. Individual core and mean flux measurements for core incubation experiment #4 conducted during June 2002.....	30
Table 6. Concentrations of (a) total dissolved Zn and (b) Zn-complexing ligands from bottom water at time point T0 from sta. 1 and sta. 2 at each sampling time. ND -= not detectable.	32
Table 7. Fluxes of Zn-complexing ligands ($\Sigma_{L,Zn}$) for individual cores at stas. 1 and 2 in the CFE.	40
Table 8. Fluxes of TDZn in individual cores at stas. 1 and 2 in the CFE.....	41
Table 9. Fluxes of TDZn and Zn-complexing ligands in different estuarine systems.....	42
Table 10. Results of resuspension experiments in the absence and presence of simulated sunlight in samples from the CFE. Triplicate water samples were 0.2 μ m-filtered with no added sediments.....	54
Table 11. Results of resuspension experiments in the presence and absence of simulated sunlight in samples from the CFE and San Diego Bay. Triplicate water samples were 0.2 μ m-filtered with added sediment (2 g wet sediment per L).	55
Table 12. Results of resuspension experiments in the presence and absence of simulated sunlight in samples from the CFE. Triplicate water samples were unfiltered with no added sediments.	56
Table 13. Results of first interlaboratory comparison for TDCu using a sample collected in July 2000 from the CFE.	66
Table 14. Results of first interlaboratory comparison for dissolved Cu speciation using samples collected in July 2000 from the Cape Fear estuary and San Diego Bay.	67
Table 15. Results of second interlaboratory comparison for total dissolved Cu and Cu speciation using a sample collected in January 2001 from San Diego Bay.	69
Table 16. Results of second interlaboratory comparison for total dissolved Zn using a sample collected January 2001 in San Diego Bay.....	69
Table 17. Results of dissolved organic carbon analyses in an ultrafiltration experiment at three sites in the Cape Fear estuary, October 2000.....	71
Table 18. Results of analyses of total dissolved Cu (TDCu) and strong Cu-complexing ligands ([L]) in an ultrafiltration experiment at two sites in the Cape Fear estuary, October 2000... .	71
Table 19. Benthic fluxes of strong Cu-complexing ligands ($\Sigma_{L,Cu}$) and TDCu from cores collected in March 2003 from station 27 located at the head of San Diego Bay.....	73

List of Abbreviations and Acronyms

α_{MAL}	Side-reaction coefficient of a metal (M) with respect to an added ligand (AL)
APDC	Ammonium pyrrolidinedithiocarbamate
ASV	Anodic stripping voltammetry
CCC	Criterion continuous concentration
CDOM	Colored (or chromophoric) dissolved organic matter
CFE	Cape Fear estuary (southeastern North Carolina)
CLE-CSV	Competitive ligand equilibration cathodic stripping voltammetry
CSV	Cathodic stripping voltammetry
DDDC	Diethylammonium diethyldithiocarbamate
DOC	Dissolved organic carbon
DoD	Department of Defense (USA)
DTPA	Diethylenetriaminepentaacetic acid
8-HQ	8-Hydroxyquinoline
EPA	Environmental Protection Agency (USA)
FEP	Fluorinated ethylene propylene (Teflon)
FHDPE	Fluorinated high-density polyethylene
HDPE	High-density polyethylene
HMDE	Hanging mercury drop electrode
h ν	Light energy
GFAAS	Graphite furnace atomic absorption spectroscopy
K'	Conditional equilibrium constant
$K'_{\text{Cu}-\Sigma L_i}$	Conditional equilibrium constant for Cu- $\Sigma L_{i,\text{Cu}}$ complexes
$K'_{\text{Zn}-\Sigma L_i}$	Conditional equilibrium constant for Zn- $\Sigma L_{i,\text{Zn}}$ complexes
kDa	Kilodalton
L, L_1, L_2, L_3	Metal complexing ligand classes
μM	Micromolar (10^{-6} M)
nm	Nanometer (10^{-9} m)
nM	Nanomolar (10^{-9} M)
NIST	National Institute of Standards and Technology
ODU	Old Dominion University
PAR	Photosynthetically available (or active) radiation
POM	Particulate organic material
PVC	Polyvinylchloride
$\Sigma L_{i,\text{Cu}}$	Sum of concentrations of copper-complexing ligands detected using a specified method
$\Sigma L_{i,\text{Zn}}$	Sum of concentrations of zinc-complexing ligands detected using a specified method
SPAWAR	Space and Naval Warfare Systems Command (US Navy)
TDCu	Total dissolved copper
TDZn	Total dissolved zinc
UNCW	University of North Carolina at Wilmington
USACE	US Army Corps of Engineers

UV
UWM

Ultraviolet
University of Wisconsin at Madison

Acknowledgements

Dr. Robert J. Kieber was co-principal investigator for this project. Dr. William J. Cooper assisted in the preparation of the initial SERDP proposal. Dr. Robert F. Whitehead (former postdoctoral research associate, now permanent researcher at UNCW) oversaw most of the project management and performed a large amount of the research for this project. His efforts were critical to the success of this project.

The following individuals performed major portions of the research described in this report: Dr. G. Christopher Shank (former Ph.D. student at the University of North Carolina at Chapel Hill, currently at US EPA in Athens, GA); Kristie L. Lieseke (former M.S. student at UNCW); and Michelle L. Smith (former M.S. student at UNCW). Sarah Williams (former undergraduate student at UNCW) performed many analyses for this project as a summer researcher in 2002. Kenneth A. MacGillivray (former M.S. student at UNCW) assisted with field sampling and worked on a separately-funded project that had relevance to this project. A number of undergraduate students, working with our Marine and Atmospheric Chemistry Research Laboratory (MACRL) at UNCW, assisted with field sampling and laboratory work.

The invaluable efforts of Captain Dan Aspenleiter and the crew of the R.V. *Cape Fear* made our field sampling possible. We also acknowledge the Department of Chemistry and Biochemistry and the Center for Marine Science at the University of North Carolina at Wilmington for their support of this project.

Executive Summary

Copper (Cu) and zinc (Zn) have numerous sources in naval shipping, berthing, and maintenance facilities. Concentrations of these metals in estuaries and harbors can reach levels which are potentially toxic to biota. Hence, discharges and concentrations of these metals are strictly regulated by environmental quality agencies. Because of pervasive problems associated with discharges and remediation of Cu and Zn in estuaries and harbors, it is essential that regulated agencies and regulators alike understand the factors that influence the availability and cycling behavior of these metals in environments relevant to DoD operations. The statement of need to which this project responded expressed the goal of an improved understanding of the *in situ* physical and chemical processes that may affect Cu and Zn speciation (forms of occurrence), potential bioavailability, and fate and cycling in estuarine waters.

The goals of this proposed study are (1) to quantitatively determine water column concentrations and benthic fluxes of total dissolved Cu, total dissolved Zn, dissolved organic carbon, and Cu- and Zn-complexing ligands seasonally at two contrasting sites in the Cape Fear estuary (CFE) in southeastern North Carolina which are representative of potentially contaminated DoD estuarine harbor facilities; (2) to determine changes in the cycling, fate, and organic speciation of dissolved Cu and Zn which may occur during resuspension events (wind and wave mixing, shipping operations, dredging), in particular focusing on the role of photochemical reactions; and (3) to examine the effects of a large scale dredging and straightening project (which began in the Cape Fear Estuary in 2001, approximately midway through our project) on the speciation, fate, and cycling of Cu and Zn in estuarine waters and sediments.

As one of three groups (CP-1156, CP-1157, and CP-1158) involved in SERDP-funded Cu and Zn compliance research, we performed an interlaboratory comparison of analytical methods. Excellent agreement was obtained for total dissolved Cu and Cu speciation methods. Good agreement was also obtained in a much less comprehensive total dissolved Zn intercomparison. No intercomparison was obtained for Zn speciation because ours (CP-1157) was the only group to perform extensive Zn speciation measurements.

In intensive seasonal studies of the CFE, concentrations of total dissolved Cu (TDCu; ~3-25 nM) and Zn (TDZn; ≤61 nM) were always well below EPA criterion continuous concentrations (CCC) in freshwater and saltwater. The relatively low concentrations of the metals in the CFE probably reflect relatively small inputs of these metals coupled with the rapid flushing rate (short residence time) of the estuary. The large excess of very strong Cu-complexing ligands ($\Sigma L_{i,Cu}$; 7 to >200 nM) relative to TDCu (~3-25 nM) maintains very low concentrations of free Cu^{2+} ($<10^{-13}$ M). The CFE system can therefore be considered to be very well “buffered” with respect to free Cu^{2+} ion. Humic substances can account for virtually all the strong Cu-complexing capacity in the CFE. Since humics are a relatively consistent fraction of the total dissolved organic carbon (DOC) in the system, and DOC behaves conservatively in the CFE, we observed a strong correlation between $\Sigma L_{i,Cu}$ and DOC. This empirical relationship can be used in the CFE to predict concentrations of $\Sigma L_{i,Cu}$ based on measurements of DOC. This is significant because the analysis of ligand concentrations is time-consuming and difficult, whereas DOC measurements are relatively simple. If this relationship can be established in other

estuarine systems containing varying levels of DOC, TDCu, and strong Cu-complexing ligands, then DOC may become a useful proxy measurement for Cu ligands. Measurements of TDCu and DOC may therefore be used to relatively quickly estimate Cu speciation (including free Cu^{2+} concentrations) in estuarine or harbor systems.

Concentrations of TDZn ranged from non-detectable (ND) to 61 nM in the upper CFE and ND to 61 nM in the lower estuary. Concentrations of Zn-complexing ligands ranged from ND to 102 nM throughout the estuary whereas TDZn concentrations ranged from ND to 61 nM. In certain samples, concentrations of Zn-complexing ligands were less than TDZn, suggesting that TDZn can titrate out relatively strong ligands leaving the remaining Zn as either weakly complexed Zn or inorganic Zn. In contrast to the large extent of organic complexation of TDCu (>99%), only 25-84% of the TDZn occurs as relatively strong organic complexes in the CFE. This differing behavior is mainly a consequence of the much weaker nature (lower K') of the Zn-ligand complexes, as well as the comparatively lower concentrations of Zn ligands relative to TDZn in the CFE. In contrast to the findings for Cu, extracted humic substances from the CFE did not significantly complex TDZn; thus humics are not a significant source of relatively strong Zn-complexing ligands in the CFE. Therefore, the ligands complexed to Zn and Cu are different and factors favoring strong Cu complexation in the CFE, and perhaps other estuarine and harbor systems, do not necessarily favor Zn complexation.

Benthic fluxes of TDCu and TDZn were generally small and sporadic in the CFE system and did not contribute significantly (<5%) to observed standing stocks of these metals in the estuarine water column. This behavior presumably reflects relatively low concentrations of these metals in the sediments and porewaters. The short residence time of water in the system may serve to keep the sediment well-flushed, thereby precluding the accumulation of dissolved metals in the porewaters.

Benthic fluxes of strong Cu-complexing ligands were generally sporadic. A relatively small number of the total measurements showed significantly large outward fluxes of ligands. Since the effluxing ligands were not analytically distinguishable from those in the water column, it is possible that some of the ligands found in the CFE water column are derived from sediment porewaters. However, given the large ligand concentrations found in the CFE water column and the short residence time of the system, benthic fluxes did not contribute significantly (<5%) to observed standing stocks. In estuarine systems possessing lower water column ligand concentrations and longer residence times (e.g., Chesapeake Bay), benthic fluxes of comparable magnitude could be a potentially significant source of strong Cu-complexing ligands.

Direct measurements of benthic fluxes of Zn-complexing ligands described in this study are the first ones reported. Similarly to fluxes of Cu ligands, Zn ligands fluxes were sporadic and contributed <5% to the total standing stock of ligands in the CFE water column. However, effluxing Zn ligands were analytically indistinguishable from those found in the water column; therefore, some of the water column ligands may be derived from sediment porewaters.

Experiments in which inorganic Cu^{2+} was added to natural CFE water and exposed to natural or simulated sunlight demonstrated that the magnitude of photobleaching or

photochemical degradation of colored dissolved organic matter (CDOM) was significantly reduced compared to experiments with no added Cu^{2+} . It is proposed that this photoprotection results from the formation of strong Cu complexes which act as a sink for superoxide radicals which would otherwise be available for photobleaching of CDOM. In the CFE, we calculated that up to 6% less photobleaching of CDOM occurs due to the ambient concentration of strongly complexed Cu. Thus, in the CFE and other estuarine and harbor systems, strongly complexed Cu may alter the degree of photobleaching in waters exposed to sunlight.

Sunlight irradiation of natural CFE waters showed that 80-95% of the initial concentration of strong Cu-complexing ligands were photodegraded in full spectrum light and ~60% was degraded by photosynthetically available radiation (PAR) over the course of 4 days. The effectiveness of PAR in photodegrading ligands is significant because PAR can penetrate up to a few meters in even darkly colored estuarine waters. The overall importance of ligand photodegradation in natural waters depends upon many factors, including light intensity, depth of light penetration, and the physics of mixing processes. However, these observations suggest that photodegradation may be a significant sink for strong Cu-complexing ligands over the relatively long term.

Sunlight irradiation of complexed Cu produced significant concentrations of dissolved Cu(I), presumably by a ligand-to-metal charge transfer process. The Cu(I) produced was probably also strongly complexed, although this could not be explicitly determined using current speciation methods. Transformations of Cu(I) and Cu(II) should show a strong diurnal cycle in estuarine surface waters, similar to what has been observed previously by other workers in oceanic surface waters. Concentrations of dissolved Cu(I) in estuaries may therefore comprise a significant but variable fraction of TDCu in estuarine waters. The monitoring of Cu(I) in sunlight irradiations of unfiltered CFE waters and resuspended bottom sediments demonstrated that ambient suspended particles and sediments either have no impact on Cu(I) production or cause a net uptake/oxidation of Cu(I). Thus resuspended sediments and ambient particles may be a net sink of Cu(I) in estuarine waters either through absorption or increased oxidation.

Resuspended natural CFE bottom sediments exposed to simulated sunlight did not appear to release or take up significant concentrations of TDCu, although some variable release was found after irradiation of unfiltered CFE water containing natural suspended particles but no bottom sediments. This suggests that the CFE suspended material, which probably contains more organic matter, may be more photoreactive than the bottom sediments. Resuspended San Diego Bay sediments, which contained much more Cu (2300 nmol Cu/g dry sediment) than the CFE sediments (3-20 nmol Cu/g dry sediment) showed a very large increase in total Cu concentration after sunlight irradiation. Similarly high release after photolysis was also observed in relatively more contaminated CFE sediments adjacent to the Port of Wilmington (50 nmol/g dry sediment). Results of the photolysis experiments using the San Diego Bay and Port of Wilmington sediments clearly indicated that photolytic release of Cu is only significant in sediments with relatively high Cu concentrations (such as San Diego Bay). Throughout the CFE, percent organic carbon was highly correlated with the amount of Cu in the sediments.

Only limited conclusions can be drawn regarding the specific effects of the large scale dredging and straightening project on Cu and Zn cycling in the Cape Fear estuary, since the

design of the study did not permit the type of fine-scale sampling that is necessary to adequately assess dredging effects. Our sediment results show that there are relatively low levels of total dissolved Cu and Zn in the CFE, and fluxes of these metals and their binding ligands do not significantly affect their standing stocks in the estuary. One of the major reasons for these observations is that the estuary is well-flushed, resulting in relatively short residence times (≤ 1 week) of the water. The dredging project is increasing the tidal volume of the system; this would serve to increase the flushing capability of the estuary, therefore leading to negligible effects upon Cu and Zn concentrations and speciation.

We found that resuspended sediments that were relatively high in Cu release metal when exposed to sunlight-exposed surface waters. However, we detected moderately elevated concentrations of sedimentary Cu in the CFE only at one station adjacent to the Port of Wilmington; downstream sediments showed unremarkable concentrations of Cu. Therefore, for the bulk of the mainstem estuary, it is unlikely that resuspension of sediments will generate Cu contamination. We cannot specifically assess the effects of resuspension from dredging of sediments in the predominantly freshwater portion of the system upstream of the Port of Wilmington; however, given the rapid flushing rate of the system, we would surmise that no significant long-term effects should be expected. We cannot assess potential Zn release from the sediments as that was not studied.

1. Objectives

The statement of need in response to which our proposal was submitted expressed the goal of an improved understanding of the *in situ* physical and chemical processes that may affect Cu and Zn speciation, potential bioavailability, and fate and cycling in estuarine waters. The specific goals of this proposed study were as follows:

- (1) to quantitatively determine water column concentrations and benthic fluxes of total dissolved Cu (TDCu), total dissolved Zn (TDZn), Cu- and Zn-complexing ligands, and dissolved organic carbon (DOC) seasonally at two sites in the Cape Fear estuary (CFE). These sites included a military shipping terminal and a commercial shipping harbor, both of which are representative of the impacts and conditions expected at typical DoD harbors.
- (2) to quantitatively determine the contribution of benthic fluxes of TDCu, TDZn, and Cu- and Zn-complexing ligands to the standing stock of these substances in overlying estuarine waters.
- (3) to determine changes in speciation, fate, and cycling of Cu which occur during resuspension events (wind and wave mixing, dredging operations), particularly focusing on the role of photochemical reactions involving Cu and DOC which may play a role in altering metal speciation and transformations. This goal included a specific determination of the photochemical conditions (light intensity and wavelength) which characterize any possible photochemically induced speciation changes in dissolved Cu and the dissolved organic complexes in which they occur.
- (4) to examine the effects of a large scale dredging and straightening project in the Cape Fear estuary (which began in 2001, approximately midway through our project) on the speciation, fate, and cycling of Cu and Zn in estuarine waters and sediments subjected to large scale disturbances that are representative of those that may occur in DoD harbors.

2. Background

2.1. Overview

The *speciation* of a dissolved trace metal refers to its distribution among various chemical forms, including free hydrated ion, inorganic complexes, and complexes with organic ligands. Speciation studies of Cu and Zn have focused almost exclusively on the water column of estuarine and oceanic environments, where organic complexation of these metals appears to control concentrations of their free hydrated ionic forms (e.g., $\text{Cu}^{2+}(\text{aq})$, $\text{Zn}^{2+}(\text{aq})$), which in turn are linked to toxicity and bioavailability of these metals. However, in the relatively shallow water environments within which DoD facilities are typically situated, sediment-water interactions are a critically important, yet commonly neglected set of processes which can affect the speciation, fate, and cycling of Cu and Zn. These interactions include fluxes across the sediment-water interface as well as resuspension of bottom sediments into overlying waters resulting from natural and anthropogenic disturbances. These factors together control the potentially bioavailable standing stock of Cu and Zn in harbors and estuaries. A thorough understanding of these processes is necessary to address compliance standards (water quality criteria, discharge regulations) that relate to environmentally realistic impacts of Cu and Zn discharges as based on potentially bioavailable metal concentrations (*metal speciation*) and not simply total metal concentrations. Information on these processes is critical for designing a scientifically-based response to regulations whose goal is to minimize toxic environmental effects in harbors and estuaries subject to shipping, loading, mooring, and associated activities such as dredging.

The main study area for this project, the lower Cape Fear River estuary in southeastern North Carolina, has a number of desirable attributes relevant to the study objectives. First, it contains a range of water types within its reaches, ranging from organic-rich coastal plain freshwaters to nearly oceanic waters. This span is representative of the range of water types comprising virtually all DoD harbor facilities. Second, the Cape Fear is undergoing a dredging and straightening project which began in 2001, approximately midway through our project, providing the possibility of assessing physical and chemical changes in metal speciation in the estuary prior to and after large scale disturbance.

This work improves our understanding of the *in situ* physical and chemical processes that may affect the speciation of dissolved Cu and Zn, and hence their potential bioavailability and toxicity in estuarine waters. We performed photochemical measurements which were a uniquely effective means for examining the mobility and transformations of photochemically labile metal complexes, which may be subject to resuspension and exposed to sunlit surface waters. This project greatly increased the very limited direct measurements of benthic fluxes of Cu and Zn and their complexing ligands, therefore allowing a better evaluation to be made of the impact of sedimentary fluxes from sediments on overlying water chemistry. An improved understanding of these processes is necessary to address compliance standards in DoD harbors that relate to environmentally realistic fates of dissolved Cu and Zn as based on potentially bioavailable metal concentrations (i.e., the fractions not complexed by organic ligands) rather than simply total metal concentrations.

2.2. Cu and Zn biogeochemistry

Dissolved trace metals in natural waters may exist as free hydrated ions (i.e., $\text{Cu}^{2+}(\text{aq})$ and $\text{Zn}^{2+}(\text{aq})$) and as complexes with inorganic and organic ligands (Donat and Bruland, 1995; Stumm and Morgan, 1996). It has been shown that the bioavailability of trace metals such as Cu and Zn to common estuarine and marine organisms (phytoplankton, copepods) is proportional to the activities of the free ionic forms of these metals and not simply to their total concentrations (Sunda and Guillard, 1976; Brand et al., 1983; Brand et al., 1986; Sunda et al., 1987, 1990). In most natural waters, organic ligands, representing a very small fraction ($\sim <0.1\%$) of the total pool of dissolved organic matter (DOM), form relatively strong complexes with these metals. Complexation of Cu and Zn by these organic ligands appears to play a significant role in regulating the biogeochemistry of the metals in natural waters by controlling concentrations of their uncomplexed, free ionic forms, which are assumed to be the bioavailable (and potentially toxic) forms to biota.

In surface oceanic and estuarine waters, dissolved Cu appears to be complexed by at least two classes of organic ligands: a very strong class (commonly termed L_1) which generally exists at concentrations ranging from 1-40 nM, with conditional stability constants (with respect to free Cu^{2+} ion, K') of 10^{12} to 10^{14} , and weaker classes (i.e., L_2 , L_3), which exist at higher concentrations (6-150 nM), but with lower values of K' (10^8 - 10^{10}) (van den Berg, 1984; Buckley and van den Berg, 1986; van den Berg et al., 1987; Hering et al., 1987; Moffett and Zika, 1987a; Sunda and Hanson, 1987; Coale and Bruland, 1988, 1990; Apte et al., 1990; Moffett et al., 1990; Sunda and Huntsman, 1991; Donat and van den Berg, 1992; van den Berg and Donat, 1992; Donat et al., 1994; Moffett, 1995). Its strong affinity for organic matter is consistent with observations that Cu is cycled with particulate organic matter (POM) in the oceanic and coastal water columns (Landing and Feely, 1981; Fischer et al., 1986; Kerner and Geisler, 1995), probably as a result of the Cu being incorporated within (Morel and Hudson, 1985) or adsorbed to the particles (Davis, 1984; Tessier et al., 1996).

In general, these metal-complexing ligands represent various fractions of dissolved or colloidal organic matter, including phytoplankton exudates and their degradation products (Anderson et al. 1984; Seritti et al. 1986; Zhou and Wangersky, 1989; Robinson and Brown 1991; Kerner and Geisler, 1995). Biogenic Cu-complexing ligands have been identified in laboratory cultures of various algae and bacteria (McKnight and Morel, 1979, 1980; Jardim and Pearson, 1984; Zhou and Wangersky, 1989; Kozarac et al., 1989; Moffett et al., 1990; also see Gledhill et al., 1997 for review). On the basis of laboratory culture experiments and field observations, the common marine cyanobacterium *Synechococcus* has been postulated as a source of the strongest Cu-complexing ligand class, L_1 (Moffett et al., 1990; Moffett and Brand, 1996). The specificity of this strong ligand even in the presence of excesses of other bioactive metals (Fe, Cd) indicates that this ligand is uniquely produced by this cyanobacterium, perhaps as a detoxifying mechanism in response to elevated levels of total dissolved Cu (Moffett and Brand, 1996). However, the chemical compositions and structures of the compounds remain unknown because of the difficulties inherent in isolating extremely small concentrations of these substances in complex matrices such as estuarine and sea water.

The speciation of dissolved Zn has been studied somewhat less intensively than Cu, primarily because there are fewer methods available for characterizing Zn-organic complexes. In the open ocean, Bruland (1989) and Donat and Bruland (1990) determined that >95% of total dissolved is strongly complexed by organic ligands having concentrations of 1.6-2.2 nM (close to the ambient Zn concentration) forming complexes with conditional stability constants (with respect to free Zn^{2+}) of 10^{10} - 10^{11} . Weaker ligand classes, with values of K' of $10^{7.4}$ to $10^{9.4}$ and with concentrations of 4-160 nM have been detected in coastal and estuarine waters (van den Berg and Dharmvanij, 1984; van den Berg et al., 1986, 1987; Muller and Kester, 1991). Calculations of organically complexed Zn in estuaries range widely from 1 to >95%. In certain polluted estuaries, total Zn concentrations appear to saturate the available organic Zn-complexing ligands, leaving an appreciable fraction of Zn in the inorganic form (van den Berg and Dharmvanij, 1984) which may increase its bioavailability and thus increase toxicity to certain organisms (Sunda et al., 1987, 1990).

Both Cu and Zn can also form both dissolved and insoluble inorganic complexes with sulfide (Luther et al., 1996). These interactions can play an important role in influencing the cycling and bioavailability of these metals in certain anoxic and hypoxic environments (Kuwabara et al., 1996; Zwolsman et al., 1997). The precipitation and redissolution of metal sulfide phases has been shown to be important in anoxic sedimentary environments (Huerta-Diaz and Morse, 1992; Morse and Arakaki, 1993; Cooper and Morse, 1998) and in highly polluted estuaries with long water residence times (Zwolsman et al., 1997).

2.3. The role of sediments in Cu and Zn speciation

The presence and transfer of metals such as Cu and Zn are of concern because toxic effects may result when the bioavailable forms of these metals are assimilated by organisms such as phytoplankton and benthic organisms, and by the higher trophic levels which feed on these organisms (Sunda and Huntsman, 1983, 1996; Cain and Luoma, 1985; Sunda et al., 1987). Many studies have demonstrated that sediment pore waters can be enriched in various metals relative to overlying waters (Elderfield et al., 1981a; Emerson et al., 1984; Shaw et al., 1990), suggesting that sediments may be a source of metals to overlying waters. Measurements of metal fluxes have been presented in many studies (e.g., Elderfield et al., 1981b; Westerlund et al., 1986; Kuwabara et al., 1996; Rivera-Duarte and Flegal, 1997). While these studies demonstrated the importance of sediments in regulating the cycling of trace metals in aquatic and marine environments, they did not take into account the speciation of the fluxing metals.

Recently, Skrabal et al. (1997, 2000) demonstrated that estuarine sediments contain large (100- to 10,000-fold) excesses of Cu-complexing ligands relative to ambient TDCu. Thus, dissolved Cu in pore waters of estuarine sediments is virtually completely complexed (96.9-99.9%) by organic or mixed inorganic-organic (i.e., sulfide-containing) ligands. Direct measurement in estuarine sediments indicated that fluxes of Cu-complexing ligands exceeded fluxes of TDCu by 3 to >40-fold, suggesting that any Cu fluxing from the sediments was likely to be organically complexed. The large concentrations of Cu-complexing ligands maintained very low free hydrated Cu^{2+} concentrations (<1 to 300 pM; Skrabal et al., 2000), thereby potentially ameliorating its bioavailability and toxicity. An additional observation of this study

was that a significant fraction, perhaps up to 50%, of the total ligand pool fluxing out of the sediments formed very strong Cu complexes (with values of K' , the conditional stability constant with respect to free Cu^{2+}) of about 10^{15} . Previous field and laboratory observations suggested that strong Cu-complexing ligands (with $K' > 10^{13}$) may be produced by cyanobacteria such as the ubiquitous coastal and oceanic species, *Synechococcus* (Moffett et al. 1990; Moffett and Brand 1996). On the basis of preliminary experiments (Skrabal et al., 1997), the K' of the strong Cu-complexing ligand class fluxing from Chesapeake Bay sediments was consistent with that of the strong ligand class found in the overlying waters, although the relative importance of the sediment source and the phytoplankton source remains to be determined and may vary among different sediment and water environments.

Zn complexation in the pore waters of various estuarine sediments has been examined by van den Berg and Dharmvanij (1984). Their results show that pore waters contained 16 to 2,000 nM of Zn-complexing ligands with values of K' of $10^{7.6}$ to $10^{9.3}$. These large ligand concentrations caused a large fraction (typically >90%) of TDZn in pore waters to exist as organic complexes. In general, the pore water ligand concentrations were much greater than overlying water concentrations, suggesting the possibility of a flux of these ligands from estuarine sediments. However, no direct flux measurements for Zn-complexing ligands have been reported in the literature.

The limited data on metal speciation in estuarine pore waters demonstrate that sediments may be a significant source of the ligands that appear to play such an important role in regulating metal speciation, and by extension, metal bioavailability and potential toxicity, in shallow water environments such as estuaries. The general applicability of these results to other estuaries has not been established. Information regarding fluxes of Cu and Zn complexing ligands in estuarine environments is critical for understanding the role of sediment-water exchange in affecting the speciation and potential bioavailability of Cu and Zn in overlying estuarine waters, particularly in impacted harbors where there may be high levels of these metals.

2.4. Cycling and transformations of Cu and Zn and their complexing ligands

The distribution, speciation, and behavior of Cu and Zn in overlying waters can be affected by processes which occur in the water column itself and by interactions with metals in bottom sediments. Water column processes include chemical transformations as a result of biological processes, including the production of metal-complexing substances by phytoplankton (McKnight and Morel, 1979, 1980; Moffett et al., 1990; Moffett, 1995; Moffett and Brand, 1996; Robinson and Brown 1991), production of biogenic degradation products which complex metals (Anderson et al. 1984; Seritti et al. 1986; Kozarac et al., 1989; Zhou and Wangersky, 1989), and the scavenging and release of Cu and Zn by redox-active Fe-Mn oxyhydroxide phases, sulfides, and POM (Kerner and Geisler, 1995; Cooper and Morse, 1996; Tessier et al., 1996). Interactions with “undisturbed” bottom sediments can occur as diffusive (Fickian transport; Berner, 1980) and advective (e.g., bioirrigation; Aller, 1982) fluxes between pore waters and overlying waters. In disturbed sediments, exchanges can occur during sediment resuspension, which commonly occurs in harbors and estuaries as a result of wind, wave, and tidal stirring, shipping and berthing activities (e.g., propeller wash), and dredging. Resuspension can engender a number of direct

and indirect effects on the speciation, concentration, and potential bioavailability of Cu and Zn in overlying waters. Direct changes can occur by the dispersion of sediment pore waters, which are typically enriched in total metals, metal-complexing organic and inorganic (e.g., sulfide) ligands relative to bottom waters, leading to increases in concentrations of these constituents in overlying waters. Indirect changes can occur in several ways, including: 1) the release of Cu and Zn from oxidizing sulfide phases present in anoxic or hypoxic sediments; 2) scavenging of dissolved Cu and Zn in overlying waters by resuspended Fe and Mn oxyhydroxides and POM; 3) indirect or direct transformation of photoactive sedimentary components (including POM and Fe and Mn phases) in surface waters with the accompanying release of associated Cu and Zn.

The release of trace metals such as Cu and Zn from oxidizing sulfides has been well documented in seasonally anoxic sediments and waters (Davies-Colley et al., 1984; Cooper and Morse, 1996, 1998). However, in environments which do not typically experience water column anoxia (e.g., San Francisco Bay; Kuwabara et al., 1996), sulfide concentrations are likely to be variable, transient and in low concentrations relative to both dissolved metal and metal-complexing ligand concentrations, thereby limiting their potential for significant control of trace metal speciation. Because our proposed study area, the CFE, does not experience water column anoxia and rarely even hypoxia (Mallin et al., 1998), sulfide is likely to be a relatively unimportant ligand for Cu and Zn.

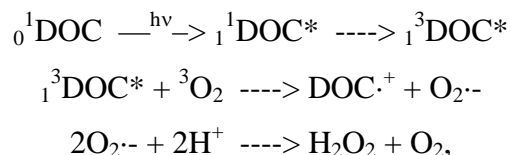
The role of particles in regulating Cu and Zn concentrations is likely dominated by interactions with POM and inorganic particles such as Fe and Mn oxyhydroxides. Rapid desorption of trace metals from oxyhydroxide particles might be expected to occur under rapidly changing pH or ionic strength conditions or during dissolution of these phases (Lion et al., 1982; Young and Harvey, 1992). However, under short time scales in estuaries, numerous studies have indicated that interactions with POM (including living and decaying phytoplankton, organically coated particles) probably dominate particle-water interactions of trace metals such as Cu and Zn (Heggie et al., 1987; Gerringa, 1990; Lee and Fisher, 1992; Fisher and Went, 1993; Kerner and Geisler, 1995). These observations are consistent with the metal-complexing ability of organic exudates and other biogenic substances as described earlier.

Of all the potential effects on metal cycling and speciation that may occur as a result of sediment resuspension, the role of photochemical transformations in estuaries is the least studied. However, several studies have indicated the role that such transformations may play in significantly altering the speciation of Cu. For example, photochemical degradation has been cited as the mechanism by which Cu(II)-organic ligand complexes in the ocean are dissociated to Cu(I) and oxidized ligand, with subsequent reoxidation of the Cu(I) to form inorganic Cu(II). Evidence for this mechanism was partly provided by Moffett and Zika (1988), who consistently observed significant concentrations of Cu(I) and H_2O_2 (which is photochemically produced from light-absorbing organic matter; Cooper et al., 1988) in oceanic surface waters during daylight hours. Decreasing concentrations of Cu(I) with depth during daylight hours and little or no Cu(I) and H_2O_2 production in the absence of sunlight suggested a photochemical origin of these species. Photochemical reactivity may also affect the cycling of the strongest Cu-complexing ligand class (L_1). Both Coale and Bruland (1988) and Moffett et al. (1990) found lower L_1 concentrations in surface waters relative to deeper waters within the photic zone, suggesting relatively high lability possibly induced by photochemical reactivity.

Organic Zn complexes may be subject to photochemical degradation also, although there have been no studies to examine this possibility. The strongest Zn-complexing ligands show a conservative distribution with depth in the photic zone of oceanic waters, suggesting a relatively higher degree of stability (Bruland, 1989). Furthermore, photoreduction of Zn(II) does not occur, as Zn(I) is not a thermodynamically stable oxidation state. However, weaker Zn-organic complexes such as have been detected in estuarine waters (van den Berg and Dharmvanij, 1984; van den Berg et al., 1986, 1987) may comprise a range of ligand types, some of which may undergo degradation with the subsequent release of Zn^{2+} .

Another photochemical mechanism which may affect Cu and Zn mobility is the potential release of these metals by photoreduction of common carrier phases, including Fe(III) and Mn(IV) oxides and POM (Waite, 1990; Stumm and Morgan, 1996). Photoreduction of Mn oxides has been observed in surface oceanic waters (Sunda et al., 1983; Sunda and Huntsman, 1988; Waite et al., 1988; Waite and Szymczak, 1993). Similar reactions may occur for Fe(III) oxides in natural waters, including surface and atmospheric waters (Behra and Sigg, 1990; Faust, 1994). Typically these photoreactions occur in the presence of dissolved or colloidal organic matter with which Cu and Zn may be associated, including fulvic and humic acids (Waite et al., 1988) and organic acids such as oxalate (Zuo and Hoigne, 1992). These types of reactions might be expected to be potentially important following resuspension of estuarine bottom sediments into illuminated surface waters. Oxidic bottom sediments are generally quite rich in Fe(III) and Mn(IV) oxides which are effective scavengers of dissolved trace metals such as Cu and Zn (e.g., Murray, 1975; Tessier et al., 1996). Upon exposure to light, photoreduction may occur leading to the release of dissolved Cu and Zn to overlying waters. A similar process might occur for Cu and Zn bound to POM in resuspended bottom sediments (e.g., Davis, 1984) when they are exposed to light in surface waters.

An additional consideration of the interaction of light with metals is the role of photochemically produced reactive species, principally H_2O_2 , which may interact with the cycles of Cu and Zn and also affect their speciation and fate in estuarine waters. The formation of H_2O_2 is considered to occur as a result of the action of sunlight (at wavelengths < 400 nm) on colored DOC via the following proposed series of reactions (Cooper et al., 1989; Sturzenegger, 1989):



where ${}_0^1\text{DOC}$ is ground state DOC, ${}_1^1\text{DOC}^*$ is excited singlet state DOC, ${}_1^3\text{DOC}^*$ is excited triplet state DOC, ${}^3\text{O}_2$ is triplet state O_2 , and $\text{O}_2^{\cdot-}$ is the superoxide radical (which also plays an important role in redox transformations). The H_2O_2 thus produced may act both reductant and oxidant for metals. Oxidation of reduced metals such as Fe(II) and Cu(I) by H_2O_2 in seawater has been reported by Moffett and Zika (1987b). Photoreduction of Fe(III) and Mn(IV) in the presence of organic matter is probably also mediated by H_2O_2 (Sunda et al., 1983; Hong and Kester, 1986; McKnight et al., 1988; Waite et al., 1988; Sulzberger et al., 1989; Bertino and Zepp, 1991). Reactions of H_2O_2 with organically complexed metals such as Cu(II)-ligand

complexes can result in the production of reduced metal complexes and oxidized ligands (Balzani and Moggi, 1990; Blough and Zepp, 1995). This mechanism has been proposed for the cycling of strong Cu(II) organic complexes in the ocean (Moffett and Zika, 1988) as discussed previously.

3. Materials and Methods

3.1. Sampling

Sediment and water sampling was primarily conducted at two sites in the CFE: station 1 located downstream of the Port of Wilmington and station 2 in the lower estuary downstream of the Military Ocean Terminal Sunny Point. Both sites are subject to shipping and berthing activities characteristic of DoD harbor facilities. Temporal (seasonal) variability in water-column distributions and sediment-water exchange of Cu, Zn, Cu- and Zn-complexing ligands, and DOC at each site were assessed by performing water column analyses and benthic flux determinations three times per year (spring, summer, late fall-winter). In addition, we collected bottom sediments and overlying water at each site for use in photochemical resuspension experiments. In our intensive studies of Cu speciation, we also conducted sampling at various stations located along the estuarine salinity gradient. Locations of station 1 and 2 and the transect stations are shown in Fig 1.

Water column samples at the surface (~2 m depth) and near bottom (1-2 m above the sediment surface) were collected and sequentially filtered through in-line 1 μm - and 0.2 μm -cartridge (Micron Calyx or Meissner) filters using a trace metal-clean pumping and filtration system. Samples were obtained at each site for trace metal speciation (fluorinated high density polyethylene [FHDPE] bottles), total dissolved Cu and Zn (high density polyethylene [HDPE] bottles), DOC (precombusted glass vials), and pH (HDPE). Samples were stored on ice until return to the laboratory. Undisturbed bottom sediments in the estuary were collected by a box corer deployed off UNCW's 19 m research vessel, the R/V *Cape Fear*. The box cores were carefully subcored using acrylic core tubes (~14 cm diameter, ~60 cm long), sealed at the bottom with internal polyethylene core caps and external rubber screw-tightened caps, and covered at the top with polyethylene caps. At least three cores were taken at each site. The cores were transported carefully back to the laboratory in a plastic box. At each site, unfiltered bottom water was collected by pumping into 50 L polyethylene carboys that were covered with black plastic bags.

Upon return to the laboratory, metal speciation samples were stored frozen (-20°C) if they could not be analyzed within a few days. Total dissolved Cu and Zn (TDCu and TDZn) samples were acidified to pH ~2 by addition of ultrapure 12 M HCl (Fisher Optima). DOC samples were stored refrigerated until analysis. Measurements of pH were made using standard methods after calibration of the meter with NIST-traceable buffers.

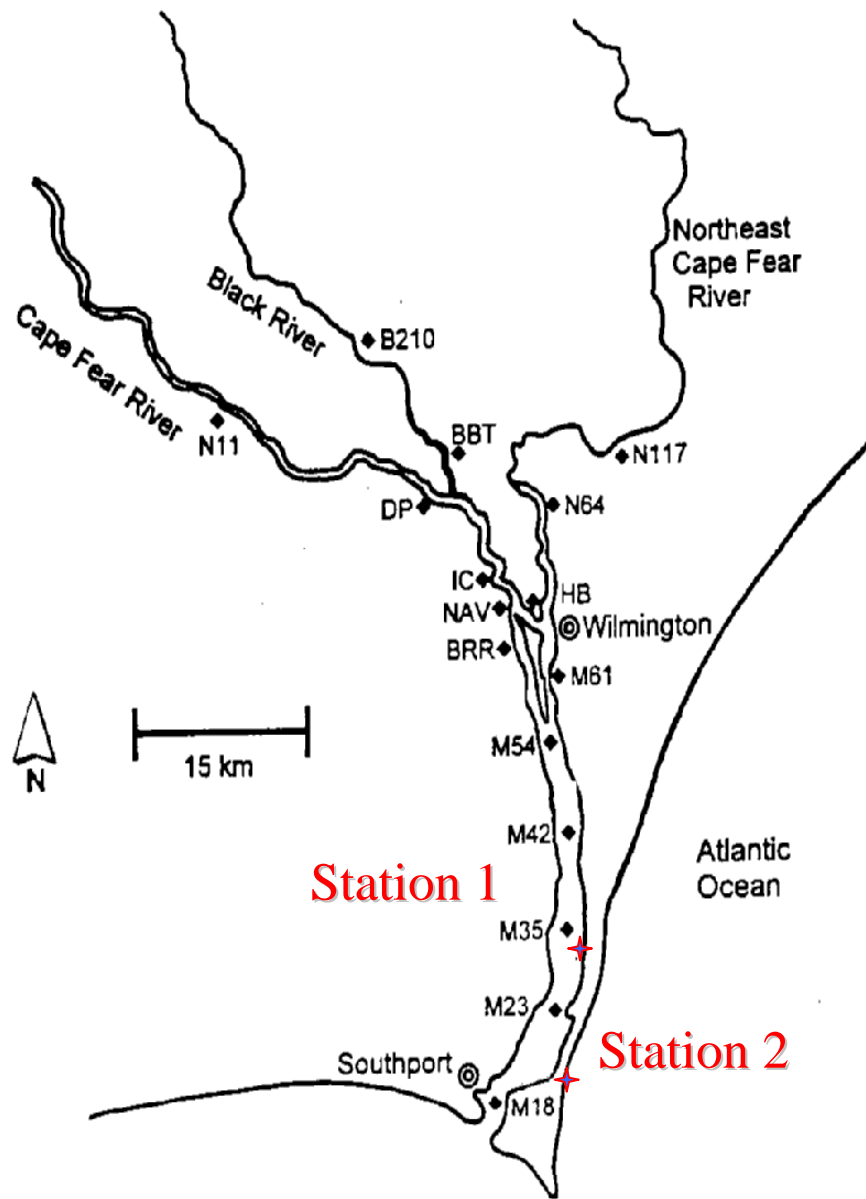


Figure 1. Map of sampling sites in Cape Fear estuary. Stations 1 and 2 were primary sampling sites for benthic flux studies. Transects were also sampled between stations M18 and M61.

3.2. Benthic flux experiments

Benthic flux experiments (using three cores from each site) were performed in an environmental chamber at ambient temperatures and light levels (i.e., darkness). Before beginning a flux experiment (typically within 24 h of return to the laboratory), the water overlying the cores was flushed using a peristaltic pump with ~3 volumes of ambient (recharge) water which is collected at the same time as the cores and stored in a polyethylene carboy under the same light and temperature conditions as the cores. The water level in the cores was adjusted to 8-13 cm above the sediment surface, with a slight space left between the overlying water level and the core top. The volume of water overlying the core was then approximately 1.2 to 1.9 L. Filtered, humidified air was gently bubbled into the water overlying the core using small diameter Teflon tubing inserted through the cover plate. The air gently mixed the water and maintained its O₂ concentration at near-ambient levels, with negligible loss of CO₂ (Burdige and Homstead, 1994).

Samples of water (~200 mL) overlying the core were removed as a function of time (generally once daily for 4-5 d and filtered in-line (0.2 µm) using a peristaltic pump. Withdrawn volumes were replaced by equal volumes of recharge water from the carboys. Analyte concentrations in the recharge water were monitored during the course of the experiment to account for any possible changes occurring during storage. Subsamples were removed for metal speciation (FHDPE bottles), total dissolved metals (HDPE bottles), and DOC (precombusted glass vials). Subsamples for speciation analyses were stored in FHDPE bottles at -20°C if they could not be analyzed in a timely manner or refrigerated for short-term storage. Aliquots for total dissolved metal analysis were acidified to pH ~2 with ultrapure HCl. DOC samples were stored refrigerated until analysis.

Fluxes of analytes were determined by linear least squares fitting of the concentration *vs.* time data, taking into account any changes occurring in the recharge water. These corrected data were converted into benthic fluxes by considering both the volumes of the water overlying the cores and the surface area of the core tubes. The validity of this technique for measuring the fluxes of Cu, Cu-complexing ligands, and DOC has been demonstrated in previous studies (Burdige and Homstead, 1994; Skrabal et al., 1997). Fluxes measured using the core incubation technique result from diffusive or bioturbation processes. Advective and turbulent processes and hydrostatic influences such as tidal pumping are not taken into account. Core incubation has the advantage of directly measuring net fluxes, including the effects of processes occurring at the sediment water interface.

3.3. Photochemical experiments

Three high density polyethylene cups were filled with surface sediment (2-3 cm depth) from the box core taken at each site and homogenized into one sample for each respective site to use for photochemical experiments. Controlled photolysis experiments were performed with 0.2 µm filtered bottom water, unfiltered bottom water, and 0.2 µm filtered water plus ~2 g wet sediment/L sediment. Sub-samples were apportioned into six 500 mL quartz flasks, whereas two aliquots were stored for analysis of initial concentrations of TDCu, Cu-complexing ligands,

Cu(I), and DOC. Three quartz flasks were enclosed in black polyethylene bags to serve as dark controls. The three light flasks were placed in a constant temperature water bath (set at ambient estuarine temperature) and irradiated in sunlight for 9 hours using a Spectral EnergyTM solar simulator (1 kW Xe arc light source) with AM1 filter to remove wavelengths not found in the solar spectrum. At the end of the experimental period the 3 dark and 3 irradiated light flasks were filtered separately through Meissner Stylux 0.2 μm polyethersulfone acid-washed capsule filters at the end of the experiment and individually analyzed for TDCu, Cu-complexing ligands, Cu(I), and DOC to determine photochemically-induced changes in these analytes for triplicate samples.

3.4. Metal speciation

To determine the speciation of dissolved Cu and Zn, we used electroanalytical methods which have been used successfully in previous work. As is discussed in previous work, electroanalytical methods can provide estimations of the concentration of metal-complexing ligands, the conditional stability constants of the metal-ligand complexes, and the speciation of the metal; i.e., expressing the concentrations of organic complexes, inorganic complexes, and free hydrated ion (Ruzic, 1982; van den Berg, 1984; Donat et al., 1994).

The speciation of dissolved Cu was determined using competitive ligand equilibration-cathodic stripping voltammetry. This technique, which has been extensively used for Cu speciation studies, involves the establishment of a competitive equilibrium between Cu, the Cu-complexing ligands naturally present in the sample, and an added organic ligand whose complexing characteristics are well known. Many different competitive ligands are available for determining Cu speciation, including catechol (van den Berg, 1984), 8-hydroxyquinoline (van den Berg, 1986), acetylacetone (Moffett and Zika, 1987a), tropolone (Donat and van den Berg, 1992), salicylaldoxime (Campos and van den Berg, 1994), and benzoylacetone (Moffett, 1995). All of these ligands form electrochemically active complexes that can be reduced at a hanging mercury drop electrode; however, they differ primarily in the strength of the complexes formed with Cu.

The detection window of each of the different techniques determines the range of naturally occurring ligand strengths that can be detected with each added ligand. This detection window depends on the relative magnitudes of the strengths of the naturally occurring Cu-ligand complexes and the complexes formed by Cu with the added ligand. Thus, the range of detected naturally occurring ligands can be varied by changing the concentration of the added ligand or by choosing added ligands that form stronger or weaker complexes with Cu^{2+} (van den Berg and Donat, 1992).

As demonstrated in many previous studies (van den Berg, 1984; van den Berg et al., 1990; Donat and van den Berg, 1992; van den Berg and Donat, 1992; Donat et al., 1994), the CLE-CSV technique allows the detection of a relatively strong class of Cu-complexing ligands, with conditional stability constants, K' , on the order of 10^{13} - 10^{15} . Observations from oceanic surface waters indicate that concentrations of this very strong ligand class are sufficient to completely complex all TDCu, and control its speciation (Moffett and Zika, 1987a; Sunda and Hanson, 1987; Coale and Bruland, 1988; Sunda and Huntsman, 1991; Donat and van den Berg,

1992). In estuarine and coastal waters, however, concentrations of this strong ligand can be less than those of TDCu. In such cases, weaker Cu-complexing ligands become important in controlling Cu speciation (Donat et al., 1994). Therefore, quantification of this strong ligand class is extremely important to understanding Cu speciation in our estuarine study environment.

We chose to use the relatively strong competitive ligand, 8-hydroxyquinoline, which can be used to detect Cu-complexing ligands strengths of K' ranging from $\sim 10^{12}$ to 10^{15} . This detection window was held as constant as possible during all Cu speciation determinations so that the concentrations and strengths of Cu-complexing ligands can be compared throughout the study. The CSV-CLE method involves the establishment of a competitive equilibrium between Cu, the Cu-complexing ligands naturally present in the sample, and a competing organic ligand, 8-hydroxyquinoline (8-HQ), added to the sample. Sample aliquots (5-10 mL) were buffered at their ambient pH, then spiked with incrementally increasing concentrations of Cu and allowed to establish a new equilibrium, typically over a period of 12 h. The Cu-8HQ complexes were deposited at a hanging mercury drop electrode (HMDE), and then a negative-going potential scan was applied. The reduction current from the Cu bound to the added competing ligand 8HQ under the newly established equilibrium conditions was then measured (using square wave voltammetry) as a function of potential. Ligand concentrations ($\Sigma L_{i,Cu}$) were calculated using SYSTAT with a non-linear one ligand fit to the following equation:

$$[Cu^{2+}]/[CuL] = [Cu^{2+}]/[\Sigma L_{i,Cu}] + 1/K'_{Cu-\Sigma L_i} [\Sigma L_{i,Cu}].$$

These concentrations were calculated using a fixed $K'_{Cu-\Sigma L_i}$ of $10^{13.5}$ which represents the approximate midpoint of pCu values (12-15) determined from our voltammetric results (Voelker and Kogut, 2001). A full discussion of the analytical details and limitations of speciation calculation methods can be found in Shank (2004a).

Zinc speciation was also determined using a CLE-CSV technique using titrations as described for Cu, but utilizing a different competing ligand, ammonium pyrrolidinedithiocarbamate (APDC) (van den Berg, 1985; Donat and Bruland, 1990). Using the van den Berg-Ruzic linearization procedure, the concentration and conditional stability constant of the Zn-complexing ligands were calculated (Ruzic, 1982; van den Berg, 1984). This method has been used to determine one class of Zn-complexing ligands in oceanic and estuarine waters (van den Berg, 1985; Donat and Bruland, 1990). Both the Cu and Zn speciation and total metal determinations (described below) were performed using electroanalytical equipment which includes an Ecochemie PGSTAT12 potentiostat coupled to a E.G & G. Princeton Applied Research model 303A HMDE and model 305 stirrer or a Bioanalytical Systems CV50W potentiostat connected to a model CGME electrode stand.

3.5. Total dissolved Cu and Zn

TDCu was determined directly on acidified samples using a CSV technique employing 8-HQ as electroactive chelating ligand (van den Berg, 1986; Shank et al., 2004a). Quantification was by the method of standard additions. Samples were UV-irradiated for 4-6 h prior to analysis in Teflon beakers covered with inverted quartz beakers using a 1.2 kW Hg arc lamp system to

destroy any organically complexed Cu. Accuracy was verified by regular analyses of certified reference materials obtained from the National Research Council (NRC) of Canada, including the riverine (SLRS-4), estuarine (SLEW-3), and oceanic (NASS-5) materials. Values obtained were always within the certified 95% confidence intervals.

TDZn was determined on UV-irradiated, acidified samples using a solvent extraction technique followed by detection using graphite furnace atomic absorption spectrometry (GFAAS). This technique involves addition of the strong Zn chelators, ammonium pyrrolidine-dithiocarbamate and diethylammonium diethyldithiocarbamate (APDC-DDDC) to an aliquot of sample buffered to pH 4-4.5, extracting the complexes into chloroform, and back-extracting into dilute ultrapure HNO₃ (Bruland, 1980). Due to volume limitation, aliquots (10 mL) of irradiated, acidified sample were diluted to 50 mL with Milli-Q water (Millipore; $\geq 18 \text{ M}\Omega \text{ cm}^{-1}$) prior to solvent extraction. All procedures, including cleaning of reagents, has been described previously (Bruland, 1980). TDZn concentrations in the extracted samples were quantified by the method of standard additions on a Perkin Elmer 5100 PC Atomic Absorption Spectrometer equipped with a 5100ZL Zeeman Furnace Module and AS 70 autosampler. In order to verify the accuracy and precision of the TDZn method, the standard reference material, SLRS-4, was analyzed. Using this method, TDZn in SLRS-4 was $15 \pm 2 \text{ nM}$ ($n = 14$), which was within the acceptable range of the certified value ($14.2 \pm 1.5 \text{ nM}$).

3.6. Dissolved organic carbon

Dissolved organic carbon were determined on acidified water samples with a Shimadzu TOC 5000 carbon analyzer equipped with a ASI 5000 autosampler. This instrument was used in the Joint Global Ocean Flux Study (JGOFS) Equatorial Pacific Ocean DOC methods comparison study and found to generate very low instrumental blanks (Sharp et al., 1993; Sharp et al., 1995). The Shimadzu TOC analyzer was also desirable because it generates analytical results comparable ($\pm 7.5\%$) to two other high temperature combustion instruments (Sharp et al., 1993; Sharp et al., 1995). The collection and analysis of DOC samples employed the protocols used during the JGOFS Equatorial Pacific Ocean DOC methods comparison study. Sample storage vials were cleaned prior to use by thoroughly rinsing with Milli-Q water and muffling at 550°C for two hours or more to remove organic material.

3.7. Acid-leachable Cu in sediments

Sediments in the CFE were measured for acid-leachable Cu concentrations. Two aliquots of $\sim 1 \text{ g}$ wet sediment from each seasonal collection were placed in 50 mL plastic centrifuge tubes, 20 mL of 1 M ultrapure HCl (Fisher Optima) was added, and samples were gently shaken over night on a shaker table. The tubes were then centrifuged for 10 minutes, and 15 mL of the supernatant was withdrawn and placed in Teflon containers for analysis by GFAAS as described previously.

3.8. Sediment organic carbon and water loss

Sediments used for solar simulator experiments were analyzed for organic carbon and water loss. Drying dishes were muffled at 550°C, then kept in a dessicator to prevent water absorption. Triplicate samples were prepared with ~3 g of wet sediment per dish. The samples were put in an oven at 60°C overnight for water loss measurement. The samples were then muffled at 550°C to determine percentage mass loss, which is taken to represent the organic content of the sediment. These values were divided by the conversion factor of 1.7 to give %OC (ISO 10694 and BS7755, 1995).

3.9. Dissolved Cu(I) determination

Dissolved Cu(I) concentrations in estuarine samples from the photochemical resuspension experiments were determined using the solvent extraction technique of Moffett and Zika (1988). This solvent extraction procedure utilized the Cu(I)-specific chelator 2,9-dimethyl-1,10-phenanthroline (dmp) in concert with the strong Cu(II) chelator ethylenediamine, which prevents artifactual formation of Cu(I) during the extraction procedure. Cu(I)-dmp complexes were extracted into methylene chloride and back-extracted into a small volume of 5% HNO₃.

For each photochemical experiment, three 250-ml aliquots were 0.2 µm-filtered within 15 minutes of collection from irradiated samples and dark controls and placed into FHDPE bottles. The extraction procedure was identical to that described by Moffett and Zika (1988) except that 250 mL aliquots were used and extracted into ~2 mL final volumes, resulting in preconcentration factors of ~125. Concentrations of Cu in the extracted samples were determined using GFAAS (using instrumentation described previously) programmed with recommended conditions. Concentrations were calculated using standard additions (Kieber et al., 2004). Percent recovery of Cu(I) was not reported due to uncertain recovery during experiments. Copper (I) results are reported instead as Δ[Cu(I)], which assumes that the recovery is similar in all samples extracted.

3.10 Contamination control

To prevent contamination of our samples, all apparatus used for trace metal analyses, including core tubes, bottles, filters, and sampling and analytical implements, were rigorously acid-washed (Bruland, 1980). All sample manipulations and analyses were performed under class 100 laminar flow hoods within the clean room facility at the University of North Carolina at Wilmington.

4. Results and Accomplishments

4.1. Overview

This project addressed and accomplished all of the major objectives originally proposed. In addition, we performed additional investigations, not originally proposed, that augmented our major objectives. The major accomplishments are described below.

4.2. Total dissolved Cu and Cu speciation in the Cape Fear estuary

In concert with previous funding from North Carolina Sea Grant, we completed a comprehensive study on Cu speciation in the CFE. Much of this work is reported in two publications (Shank et al., 2004a,b) whose results are summarized in this section. Using an electrochemical technique, competitive ligand equilibration-cathodic stripping voltammetry (CLE-CSV) with 8-hydroxyquinoline (8-HQ) as competing ligand, we focused only on that portion of Cu-complexing ligands that are considered very strong (L_i -type ligands; Donat and Bruland, 1995; Bruland et al., 2000). This strength is reflected in the side-reaction coefficient of Cu with respect to added 8-HQ ($\alpha_{\text{Cu-8HQ}}$), which had a mean value of $10^{5.2}$ in our studies. Inconsistencies and problems with the use of the L_i terminology have been noted by several authors (e.g., Town and Filella, 2000; Voelker and Kogut, 2001) and mainly relate to the arbitrary nature of the ligand class distinctions. An alternative terminology, $\Sigma L_{i,Cu}$, representing the sum of Cu-complexing ligands within the experimental detection window (in this work, at a fixed conditional stability constant with respect to the Cu- ΣL_i complexes, $K'_{\text{Cu-}\Sigma L_i}$), is therefore used in the remainder of this work.

Copper speciation was examined on eleven transects of the CFE from July 1998 to September 2000, at three stations (M18, M35, M54 or M61; see Fig. 1) located in the lower, middle, and upper estuary, respectively. Relevant data and physical parameters from these transects are listed in Table 1. Concentrations of $\Sigma L_{i,Cu}$ among all transects ranged from 7 to >200 nM. These ligands were always in excess of TDCu concentrations, which ranged from 3-25 nM, and complexed >99.9% of the TDCu. As a result, concentrations of free hydrated Cu^{2+} ion were limited to concentrations of <0.03-0.25 nM.

Concentrations of DOC examined on 6 transects showed conservative distributions with respect to salinity, ranging from ~1000 to 1250 μM at the low salinity end member (salinity <3) (Fig. 2). The conservative behavior of DOC has been previously reported in the CFE (Avery et al., 2003) and reflects the relatively degraded nature of terrestrially derived organic matter entering the Cape Fear system. Concentrations of ΣL_i were highly correlated ($r^2 = 0.93$, $p \ll 0.01$) with DOC concentrations at DOC levels of <1000 μM (Fig. 3). The relationship did not hold in low salinity waters with DOC >1000 μM . Other studies have also found correlations of DOC and strong Cu-complexing ligand concentrations (Severn estuary, UK: Apte et al., 1990; Westerschelde and Oosterschelde estuaries, The Netherlands: Gerringa et al., 1996; Galveston Bay, USA: Tang et al., 2001), but none with as strong a correlation as this study.

The strong $\Sigma L_{i,Cu}$ -DOC correlation ($r^2 = 0.94$, $p \ll 0.01$) also existed in ultrafiltered fractions (<0.45 μm feed, <10 kDa, <1 kDa) of upper and lower CFE samples collected in April

2002 in cooperation with Dr. M. Shafer's group at the University of Wisconsin-Madison (SERDP project CP-1158). This indicates that the strong Cu-complexing capacity of Cape Fear waters is distributed across different size classes, including colloids, and is apparently a function of the quantity of DOC (Fig. 4). A similar relationship between strong Cu-complexing ligands and DOC in ultrafiltration fractions was observed by Tang et al. (2001) in Galveston Bay.

Humic substances, comprising a significant fraction of Cape Fear DOC, were isolated from Cape Fear estuarine waters using a solid-phase C₁₈ extraction procedure (Kieber et al., 1999). A solution containing 26 mg humics per L in deionized water was diluted using Sargasso Sea water to form a series of solutions of salinities 5, 12, 19, and 30 as a simulation of estuarine mixing. These samples were analyzed for $\Sigma L_{i,Cu}$ concentrations using approximately the same competition level ($\alpha_{Cu-8HQ} \approx 10^5$) as the Cape Fear transect samples. Concentrations of $\Sigma L_{i,Cu}$ were correlated to humic-derived DOC concentrations ($r^2 = 0.92$, $p = 0.04$), but with some variability at high DOC levels, similar to what was observed in the estuarine transects (Fig. 5). Based on previous measurements showing that ~30-50% of the DOC in the CFE is humic-based (R. Kieber, unpublished data), and using approximate estuarine DOC:humic C ratios of 3:1 and 2:1, it appears that the C₁₈-extracted humics could account for virtually 100% of the $\Sigma L_{i,Cu}$ concentrations observed in the CFE. Similar strong Cu-complexation results have been observed by Kogut and Voelker (2001) using freshwater and seawater solutions of humic and fulvic acid isolates as well as by Xue and Sigg (1999) using fulvic acid in freshwater. Our results suggest that (1) allocthonous organic matter is the primary source of strong Cu-complexing ligands to the CFE, overshadowing any possible biological sources; and (2) it may be possible to predict the strong Cu-complexing capacity of estuarine waters based on measurement of DOC, a parameter which is much more analytically accessible than $\Sigma L_{i,Cu}$.

sampling date	site	temperature	salinity	pH	DOC (μM C)	TDCu (nM)	detection window log α_{Cu8HO}	$\Sigma L_{i,\text{Cu}}$ (nM)
27-Jul-98	M18	29.2	34.9	8.0	no sample	3.2 ± 0.5	4.8	7 ± 2
	M35	29.5	28.2	7.9	no sample	4.6 ± 0.3	5.0	46 ± 5
	M54	29.8	18.5	7.7	no sample	10.2 ± 0.2	5.2	127 ± 8
	NAV	30.2	7.9	7.6	no sample	21.0 ± 1.6	5.5	122 ± 2
	NCF6	30.1	9.7	7.5	no sample	16.7 ± 1.4	5.4	149 ± 2
19-Feb-99	M18	13.6	32.7	8.0	202	4.3 ± 0.4	4.9	11 ± 2
	M35	13.2	17.7	7.8	564	6.1 ± 0.5	5.2	120 ± 16
	M54	13.1	8.4	7.6	783	6.4 ± 0.5	5.5	176 ± 30
19-Apr-99	M18	16.9	32.6	7.9	248	2.9 ± 0.4	4.9	16 ± 1
	M35	17.7	20.0	7.8	518	6.7 ± 0.8	5.1	105 ± 2
	M54	18.7	7.0	7.5	762	20.5 ± 1.8	5.5	155 ± 3
11-Jun-99	M18	25.9	33.3	8.0	214	3.4 ± 1.1	4.9	24 ± 1
	M35	27.0	25.6	7.9	416	8.3 ± 0.4	5.1	53 ± 0
	M54	27.4	15.2	7.7	700	7.5 ± 1.6	5.3	114 ± 0
	NAV	27.5	7.8	7.4	867	20.8 ± 0.7	5.3	160 ± 15
	NCF6	28.0	5.1	7.3	1153	15.6 ± 1.0	5.3	222 ± 22
17-Dec-99	M18	14.0	27.0	7.9	352	6.0 ± 0.5	5.0	36 ± 1
	M35	13.5	15.0	7.6	648	10.2 ± 0.7	5.3	110 ± 4
	M61	12.3	3.0	7.4	976	12.0 ± 0.5	5.7	229 ± 35
30-Jun-00	M18	28.3	32.0	8.0	242	3.0 ± 0.4	5.0	12 ± 1
	M35	27.8	25.7	8.0	526	6.2 ± 1.2	5.2	69 ± 14
	M61	28.3	8.0	7.6	782	18.3 ± 1.8	5.5	178 ± 19
	NAV	28.4	2.2	7.4	1336	22.7 ± 1.5	5.7	201 ± 21
	NCF6	28.9	3.0	7.3	1242	14.7 ± 2.0	5.4	164 ± 0
20-Sep-00	M18	25.5	31.0	7.9	278	3.8 ± 1.4	5.0	20 ± 7
	M35	25.3	14.7	7.8	724	10.7 ± 0.7	5.4	102 ± 10
	M61	25.2	3.3	7.4	1286	14.2 ± 1.5	5.6	197 ± 32

Table 1. Physical parameters, DOC, TDCu, and Cu speciation data for Cape Fear estuarine transects. (TDCu = mean \pm s.d., $n = 3$; $\Sigma L_{i,\text{Cu}}$ = mean $\pm 0.5 \times$ range at fixed $K'_{\text{CuL}} = 10^{13.5}$, $n = 2$). From Shank et al. (2004a).

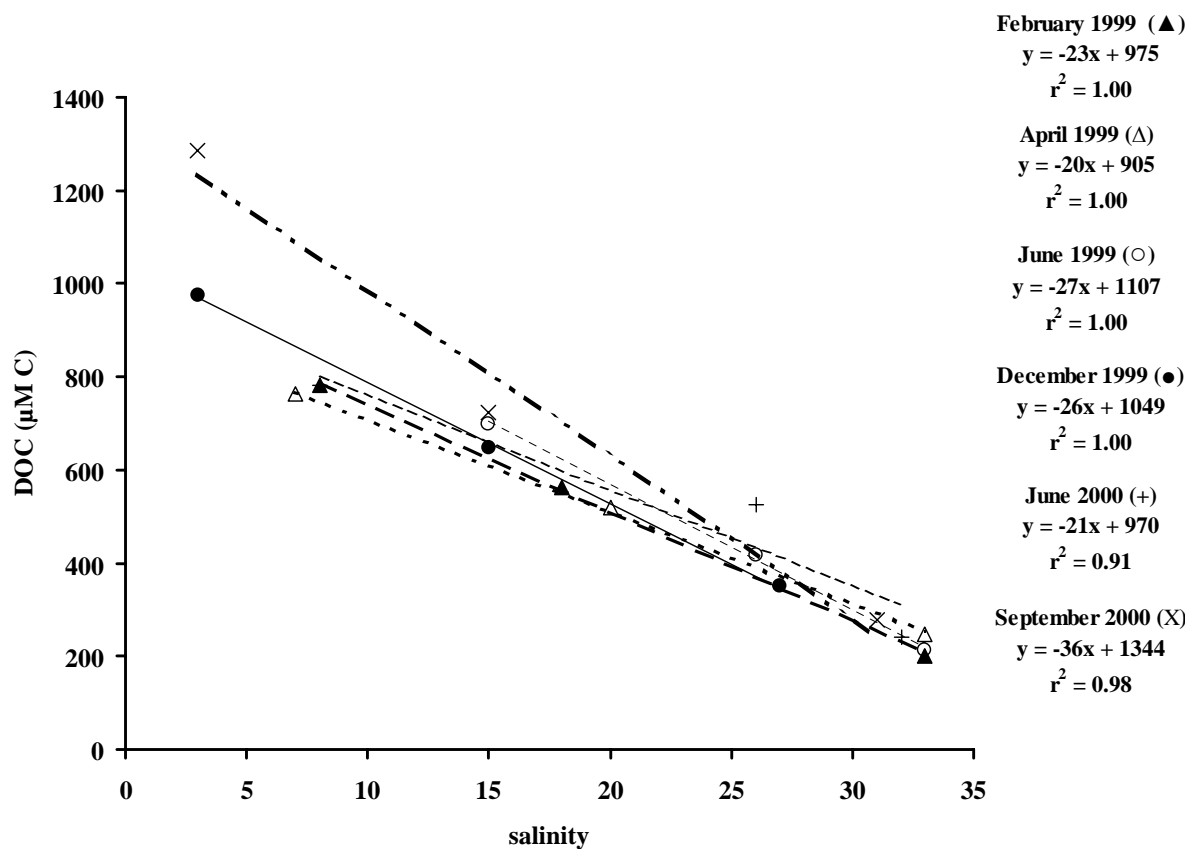


Figure 2. Distribution of DOC with respect to salinity for completed Cape Fear estuarine transects from February 1999 to September 2000. DOC data for February 1999 to December 1999 are also presented in Avery et al. (2003). From Shank et al. (2004a).

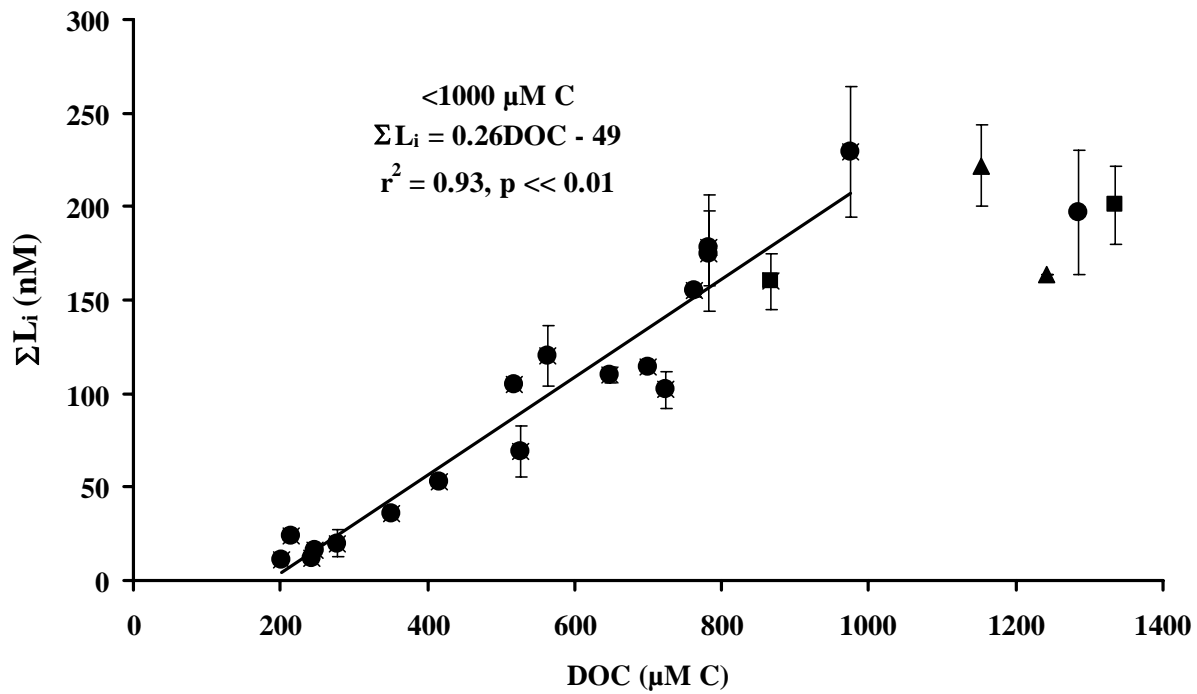


Figure 3. Relationship between $\Sigma L_{i,Cu}$ and DOC concentrations for the Cape Fear estuary (February 1999 to September 2000). M18, M35, M54/61 (●), NAV (■), NCF6 (▲). From Shank et al. (2004a).

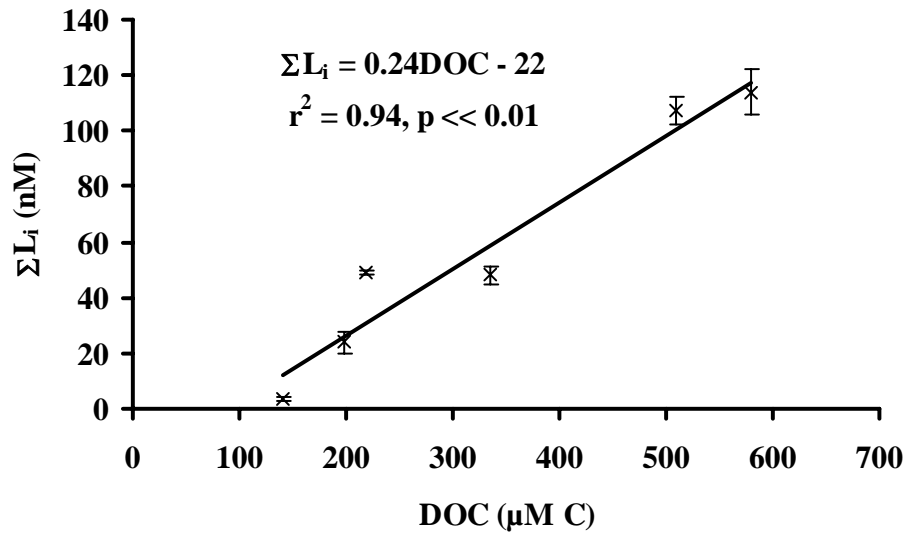


Figure 4. Relationship between $\Sigma L_{i,Cu}$ and DOC concentrations in ultrafiltered size fractions of Cape Fear estuarine water (April 2002). Feed samples were 0.45 μm filtered. From Shank et al. (2004a).

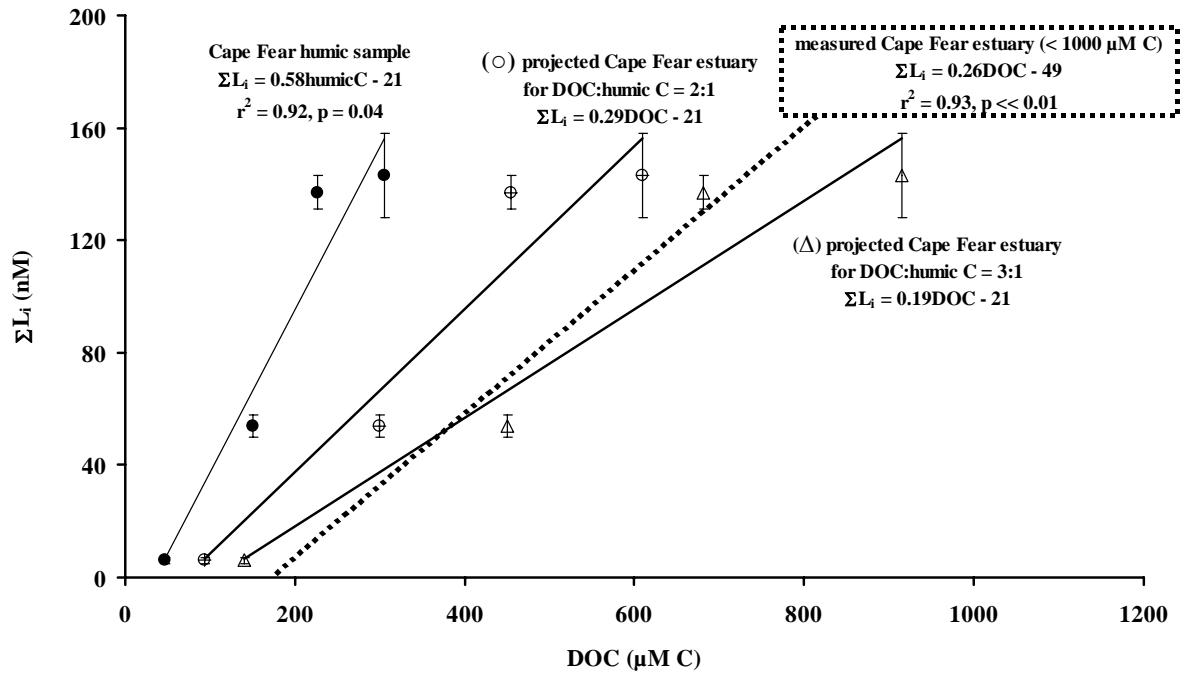


Figure 5. Comparison of $\Sigma L_{i,Cu}$ vs. projected DOC relationship for prepared Cape Fear humic solutions and $\Sigma L_{i,Cu}$ vs. DOC relationship measured for Cape Fear estuarine transect data. Projected DOC concentrations were calculated using estimated DOC:humic C ratios of 2:1 (\circ) and 3:1 (Δ). From Shank et al. (2004a).

4.3. Benthic fluxes of TDCu and strong Cu-complexing ligands

The publication by Shank et al. (2004c) describes results of our benthic flux experiments for TDCu and strong Cu-complexing ligands detectable using the CLE-CSV technique described previously. The important results of that paper are summarized here.

These experiments were performed on cores collected in April, June, and October 2001 and June 2002 at stas. 1 and 2 in the CFE (Fig. 1). Results from these experiments are shown in Table 2-5). Relatively large outward fluxes of strong ligands occurred only in sta. 2 cores during June 2001 ($340\text{--}1500\text{ nmol m}^{-2}\text{ d}^{-1}$) and in June 2002 in both sta.1 cores ($230\text{--}1400\text{ nmol m}^{-2}\text{ d}^{-1}$) and in sta. 2 cores ($0\text{--}520\text{ nmol m}^{-2}\text{ d}^{-1}$). All other ligand fluxes were either statistically insignificant, or small and inconsistent in direction. Consistently large outward fluxes of DOC occurred only during June 2002 at both sta. 1 ($0\text{--}1200\text{ }\mu\text{mol C m}^{-2}\text{ d}^{-1}$) and sta. 2 ($2000\text{--}3700\text{ }\mu\text{mol C m}^{-2}\text{ d}^{-1}$). At other times, DOC fluxes were statistically insignificant, or small ($\leq \pm 1000\text{ }\mu\text{mol m}^{-2}\text{ d}^{-1}$) and of variable direction. A marked relationship between fluxes of DOC and strong ligand occurred only in cores from stas. 1 and 2 during June 2002. In these cores, the average Cu ligand:DOC ratio of $3 \times 10^{-5}\text{ mol g}^{-1}\text{ C}$ was similar to the water column ratio of $\sim 2 \times 10^{-5}\text{ mol g}^{-1}\text{ C}$ found in the CFE by Shank et al. (2004a).

Since the ligands that do flux from the sediment are not distinguishable from those in the Cape Fear water column ($K'_{Cu-L_i} \geq 10^{13}$), it is possible that benthic fluxes may be a source of at least some of the strong Cu-complexing ligands found in Cape Fear estuarine waters. These fluxing ligands may include humic materials, biologically produced ligands (e.g., thiols), or diagenetically produced organic substances. Our calculations suggest that benthic fluxes of strong Cu-complexing ligands probably contribute $<1\%$ of the total pool of strong ligands found in the Cape Fear water column. This small contribution is attributed to the very large ambient ligand concentrations already present in the water column, coupled with a very short water residence time in the estuary (<1 week). However, comparable benthic fluxes of strong ligands that occur in estuarine systems having relatively long residence times (e.g., Chesapeake Bay, 6-12 months) may be an important source of water column ligands.

The largest TDCu fluxes were directed into the sediment ($100\text{--}210\text{ nmol m}^{-2}\text{ d}^{-1}$) at sta. 2 during June 2001. All other TDCu fluxes were either statistically insignificant, or small and of variable direction ($< \pm 70\text{ nmol m}^{-2}\text{ d}^{-1}$). Fluxes of TDCu were not related to the behavior of strong ligands. Overall, the TDCu fluxes were quite small, comparable to many other studies (see Rivera-Duarte and Flegal, 1997), and indicative of relatively uncontaminated sediments. In general, the lack of significant fluxes of both TDCu and ligands is probably attributable to the high flushing rate of the Cape Fear system, which does not allow significant accumulation of these constituents in the upper part of the sediment column where exchange is most likely to occur.

April 2001

Incubation temperature = 20 °C

Core	salinity	DOC $\mu\text{moles C m}^{-2} \text{ d}^{-1}$	strong ligand $\text{nmoles m}^{-2} \text{ d}^{-1}$	TDCu $\text{nmoles m}^{-2} \text{ d}^{-1}$
1A	13	320	0	-56
1B	13	0	0	0
1C	13	0	0	0
Mean flux		110 \pm 190	no significant fluxes	-19 \pm 32
2A	23	300	0	0
2B	23	0	380	0
2C	23	0	0	0

Table 2. Individual core and mean flux measurements for core incubation experiment #1 conducted during April 2001. Non-statistically significant ($p > 0.05$) fluxes are represented as '0' net flux. A positive flux represents an outward flux from sediment pore waters to the overlying water. From Shank et al. (2004c).

June 2001

Incubation temperature = 27 °C

Core	salinity	DOC $\mu\text{moles C m}^{-2} \text{ d}^{-1}$	strong ligand $\text{nmoles m}^{-2} \text{ d}^{-1}$	TDCu $\text{nmoles m}^{-2} \text{ d}^{-1}$
1A	12	0	0	0
	Mean flux	99 ± 170	130 ± 220	no significant fluxes
1B	12	0	380	0
1D	12	0	0	0
	Mean flux	no significant fluxes	130 ± 220	no significant fluxes

Table 3. Individual core and mean flux measurements for core incubation experiment #2 conducted during June 2001. Non-statistically significant ($p > 0.05$) fluxes are represented as ‘0’ net flux. A positive flux represents an outward flux from sediment pore waters to the overlying water. From Shank et al. (2004c).

October 2001

Incubation temperature = 17 °C

Core	salinity	DOC $\mu\text{moles C m}^{-2} \text{ d}^{-1}$	strong ligand $\text{nmoles m}^{-2} \text{ d}^{-1}$	TDCu $\text{nmoles m}^{-2} \text{ d}^{-1}$
1A	26	730	0	23
1B	26	0	0	19
1C	26	0	0	0
Mean flux		240 ± 420	no significant fluxes	14 ± 12
2A	29	0	29	0
2B	29	0	-14	53
2C	29	0	0	0
Mean flux		no significant fluxes	5 ± 22	18 ± 31

Table 4. Individual core and mean flux measurements for core incubation experiment #3 conducted during October 2001. Non-statistically significant ($p > 0.05$) fluxes are represented as '0' net flux. A positive flux represents an outward flux from sediment pore waters to the overlying water. From Shank et al. (2004c).

June 2002

Incubation temperature = 28 °C

Core	salinity	DOC $\mu\text{moles C m}^{-2} \text{ d}^{-1}$	strong ligand $\text{nmoles m}^{-2} \text{ d}^{-1}$	TDCu $\text{nmoles m}^{-2} \text{ d}^{-1}$
1A	27	3100	1000	0
1B	27	2000	230	0
1C	27	3700	1400	0
Mean flux		2900 ± 840	870 ± 580	no significant fluxes
2A	32	1200	520	-69
2B	32	1100	220	30
2C	32	0	0	-33
Mean flux		760 ± 660	250 ± 260	-24 ± 50

Table 5. Individual core and mean flux measurements for core incubation experiment #4 conducted during June 2002. Non-statistically significant ($p > 0.05$) fluxes are represented as ‘0’ net flux. A positive flux represents an outward flux from sediment pore waters to the overlying water. From Shank et al. (2004c).

4.4. Total dissolved Zn and Zn speciation in the Cape Fear estuary

Most of the work regarding TDZn concentrations and Zn speciation in the Cape Fear was completed as part of a M.S. thesis (Lieseke, 2003) and will also be presented in manuscripts for peer-reviewed publications. The following discussion is adapted from this thesis.

Concentrations of TDZn and Zn-complexing ligands ($\Sigma L_{i,Zn}$) in bottom waters at stas. 1 and 2 (Fig. 1) of the CFE were determined by analyzing the recharge water collected during core incubation experiments at time point T0. These results are shown in Table 6. The competition exerted by the ligand used in the CLE-CSV analyses ($\alpha_{ZnPDC} \approx 1$) allowed detection of ligands that form complexes with Zn with conditional stability constants, $K'_{Zn-\Sigma L_{i,Zn}}$ of $\sim 10^7$ - 10^{10} . Concentrations of TDZn ranged from non-detectable (ND) to 44 nM in the upper estuary (sta. 1) and ND to 61 nM in the lower estuary (sta. 2). Concentrations of $\Sigma L_{i,Zn}$ ranged from 10 to 102 nM in the upper estuary and ND to 67 nM in the lower estuary.

This range of concentrations is similar to that found previously for TDZn in the CFE. O'Connell (1999) found levels ranging from 15 to 90 nM throughout the estuary whereas MacGillivray (2003) reported TDZn concentrations of 5-10 nM and $\Sigma L_{i,Zn}$ concentrations of 36-96 nM at sta. 2 in the lower estuary. Values reported for the CFE are similar to previous work found in other East Coast estuaries, such as Chesapeake Bay and Narragansett Bay. Henry (1996) found concentration ranges of TDZn and $\Sigma L_{i,Zn}$ of 3-39 nM and 12-54 nM, respectively, in Chesapeake Bay. Kozelka and Bruland (1998) found TDZn and $\Sigma L_{i,Zn}$ concentrations of 16-72 nM and 11-48 nM, respectively, in Narragansett Bay. In contrast, Manila Bay, a relatively more impacted estuary, had TDZn levels ranging from 2-147 nM and $\Sigma L_{i,Zn}$ ranging from 2-123 nM, where elevated levels occurred near point sources (Vesasquez et. al., 2002). The higher concentrations observed in impacted estuaries suggests the possibility of anthropogenic inputs.

Variability of TDZn and $\Sigma L_{i,Zn}$ was observed between seasons and sampling locations in the CFE. Seasonal variability was observed in the lower estuary (sta. 2) where higher concentrations occurred in the fall compared to the spring and winter-spring. No seasonal variability occurred in the middle estuary (sta. 1). Concentrations of TDZn and $\Sigma L_{i,Zn}$ were also much greater in the spring and winter in the middle estuary compared to the lower estuary, probably reflecting the higher reactivity of the finer-grained sediments in the middle estuary.

There was no significant correlation observed between TDZn and $\Sigma L_{i,Zn}$ when data from stas. 1 and 2 were considered together. However, in considering the data separately from each station, a significant correlation was found at sta. 2 (Fig. 6b; $r^2 = 0.99$, $p \leq 0.01$) whereas there was no correlation at sta. 1 (Fig. 6a). Station 2 concentrations of TDZn and $\Sigma L_{i,Zn}$ were therefore tightly coupled. This behavior was also observed in the Scheldt estuary, where the TDZn concentrations (15-230 nM) were similar to those of $\Sigma L_{i,Zn}$ (22-220 nM). In contrast, sta. 1 concentrations were more variable, with $\Sigma L_{i,Zn}$ concentrations greater than TDZn concentrations in 4 of the 6 experiments. In 2 of the 6 experiments, $\Sigma L_{i,Zn}$ concentrations were less than TDZn, suggesting that TDZn can titrate out relatively strong ligands leaving the remaining Zn as either weakly complexed Zn or inorganic Zn. This behavior was observed in a more impacted British estuary, the Humber, in which the TDZn concentrations (91-275 nM)

Season	Sampling Date	Station 1	Station 2
Fall	November 29, 2000	19 ± 10	23 ± 1
	October 29, 2001	24 ± 3	61 ± 13
Summer	June 26, 2001	30 ± 12	43 ± 6
	June 28, 2002	ND	ND
Spring	April 23, 2001	44 ± 29	ND
Winter-Spring	March 7, 2002	31 ± 2	ND

(a)

Season	Sampling Date	Station 1	Station 2
Fall	November 29, 2000	102 ± 8	25 ± 6
	October 29, 2001	10 ± 4	67 ± 18
Summer	June 26, 2001	38 ± 23	44 ± 7
	June 28, 2002	12 ± 3	ND
Spring	April 23, 2001	31 ± 18	ND
Winter-Spring	March 7, 2002	101 ± 11	ND

(b)

Table 6. Concentrations of (a) total dissolved Zn and (b) Zn-complexing ligands from bottom water at time point T0 from sta. 1 and sta. 2 at each sampling time. ND -= not detectable.

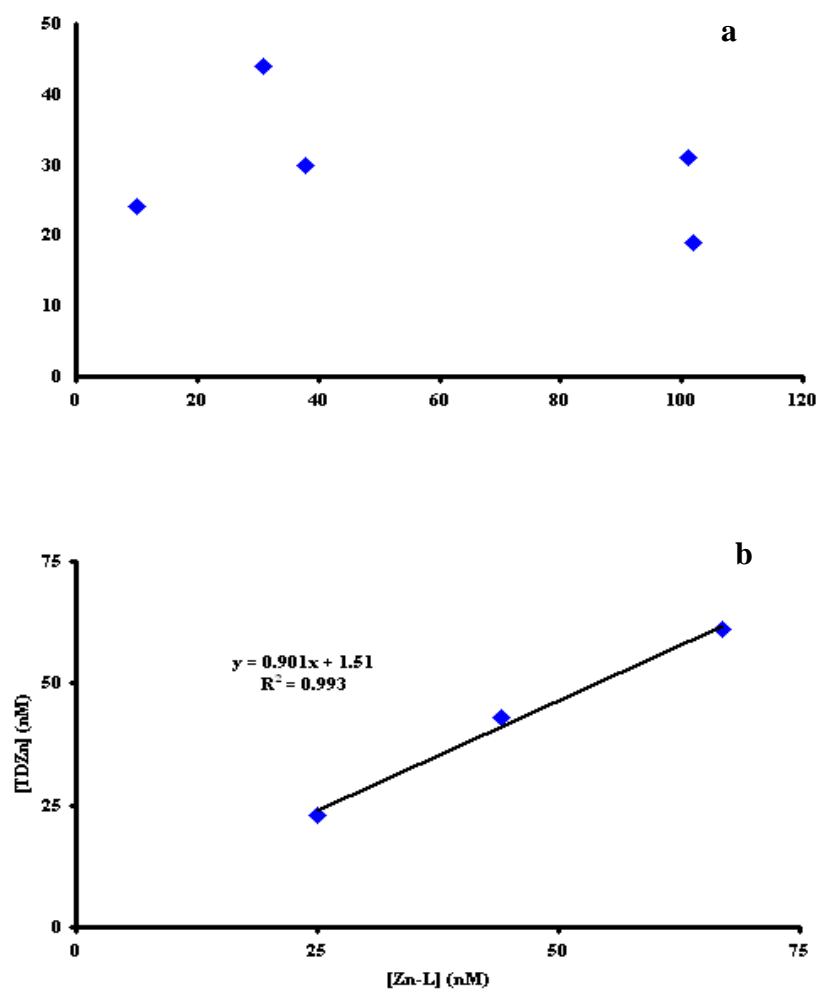


Figure 6. Relationship between TDZn and Zn-complexing ligands from bottom waters at (a) station 1 (no significant correlation; $p > 0.05$) and (b) station 2 (significant correlation; $p \leq 0.01$).

were consistently greater than $\Sigma L_{i,Zn}$ (46-275 nM). Calculations of organically complexed Zn, using the approach described in Ellwood and van den Berg (2000) showed 25-84% of the TDZn occurring in CFE as organic complexes. The degree of complexation of Zn in the CFE is generally much lower than that of Cu, of which >99.9% exists as complexed species throughout the estuary (see section 4.2). This differing behavior is mainly a consequence of the much weaker nature (lower K') of the Zn- $\Sigma L_{i,Zn}$ complexes, as well as the comparatively lower concentrations of Zn ligands relative to TDZn in the CFE. A significant fraction of dissolved Zn in the estuary therefore exists in inorganic or weakly organically complexed forms, whereas dissolved Cu exists predominantly as very strong organic complexes.

Organic-rich estuaries such as the Cape Fear may contain relatively high concentrations of metal-complexing ligands, given DOC concentrations typically range from 200-800 μ M (Avery et al., 2003). As discussed in section 4.2, concentrations of DOC are highly conservative with respect to salinity in the CFE (Avery et al., 2003). Furthermore, Shank et al. (2004a) determined that concentrations of $\Sigma L_{i,Cu}$ were highly correlated with DOC concentrations and suggested that concentrations of strong Cu-complexing ligands could be estimated from the DOC concentrations. The significant correlation between DOC and strong Cu-complexing ligands indicates that a small but significant fraction of DOC consists of Cu-complexing ligands. Concentrations of $\Sigma L_{i,Zn}$ were plotted as a function of DOC in order to determine if a significant correlation could also be observed. No statistically significant correlations occurred between DOC and $\Sigma L_{i,Zn}$ (Fig. 7). This suggests that Zn-complexing ligands comprise a more variable proportion of the DOC pool in the CFE, relative to the fraction of DOC that consists of strong Cu-complexing ligands. It is also possible that there is a class of Zn-complexing ligands that covaries with DOC, but is too weak to be detected by the current method.

Concentrations of DOC in the CFE were positively correlated to river flow (Fig. 8; $r^2 = 0.54$, $p \leq 0.01$). This relationship has been observed for other estuaries along the southeast U.S. coast (Moran et al., 1999) and suggests that DOC is flushed from upland area and adjoining soils during times of high rainfall and subsequent runoff. The impact of river flow was also examined for TDZn and $\Sigma L_{i,Zn}$ using United States Geological Survey river flow data from the Cape Fear River (available at <http://waterdata.usgs.gov/nc/nwis>). River flow was calculated by taking an average of the daily mean stream flow for the sampling date and the 6 previous days to correspond with the approximate residence time of water in the estuary (~1 week). There was no statistically significant correlation between TDZn and Zn-complexing ligands and river flow (Fig. 9). This lack of correlation suggests the importance of local inputs of Zn and ligands that are not significantly affected by river stage. This behavior differs from what has been observed for Cu-complexing ligands in the CFE because the fraction of DOC that strongly complexes Cu increases with total DOC which in turn increases with river flow (Shank, 2004a).

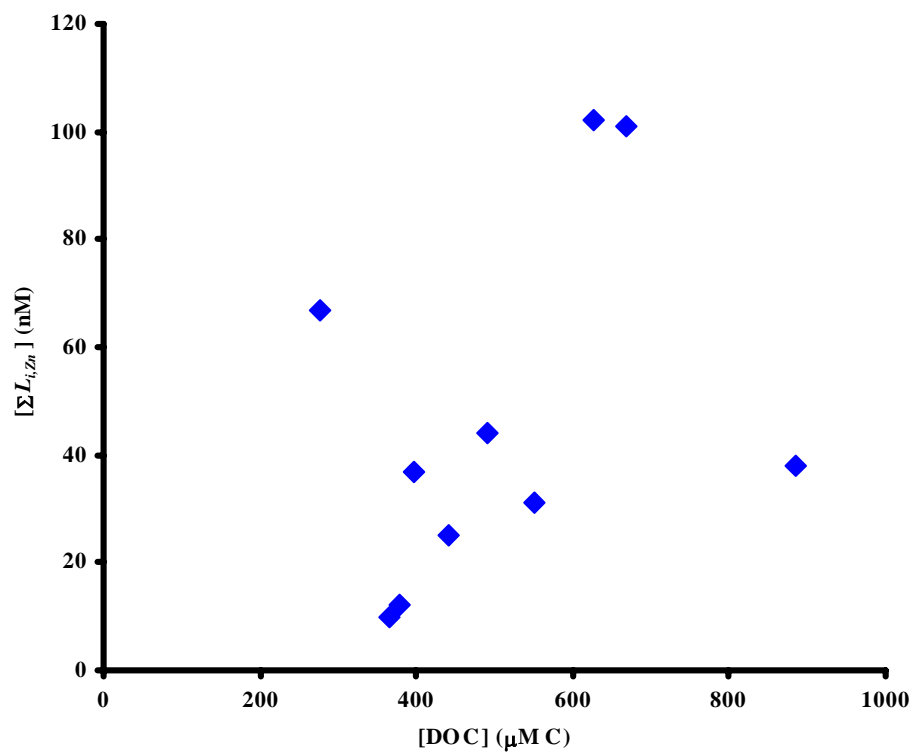


Figure 7. Relationship between strong Zn-complexing ligands and DOC for all sampling times at stations 1 and 2 in the CFE. There is no statistical significance to this relationship ($p > 0.05$).

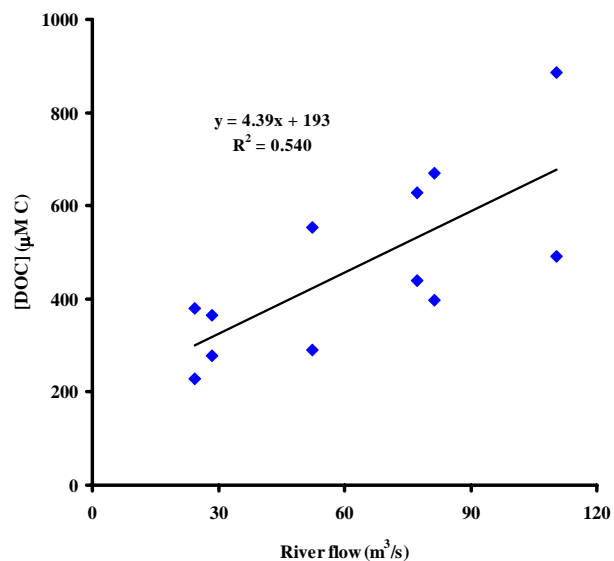


Figure 8. Relationship between DOC concentrations and river flow, showing a statistically significant correlation ($r^2 = 0.54$; $p \leq 0.01$).

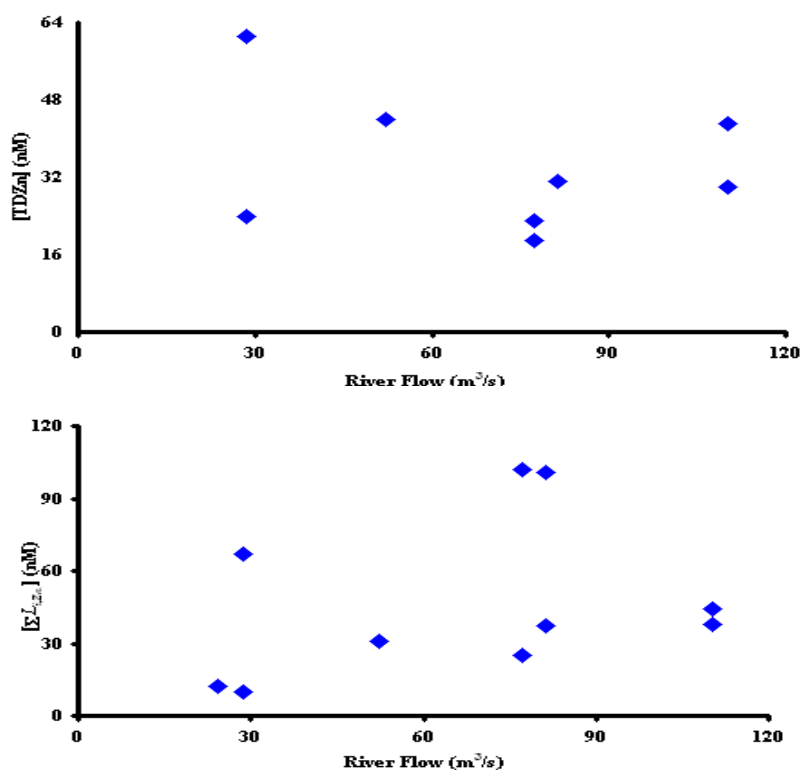
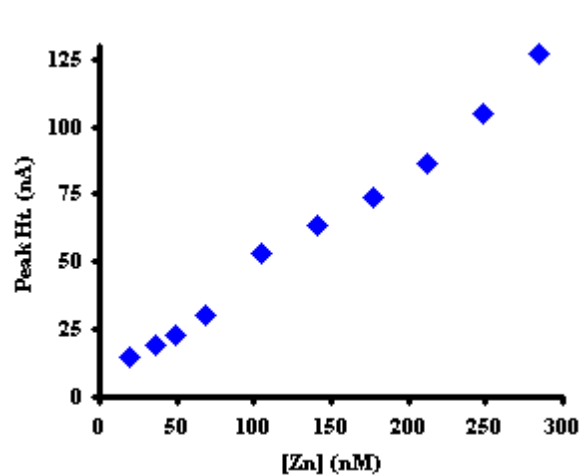
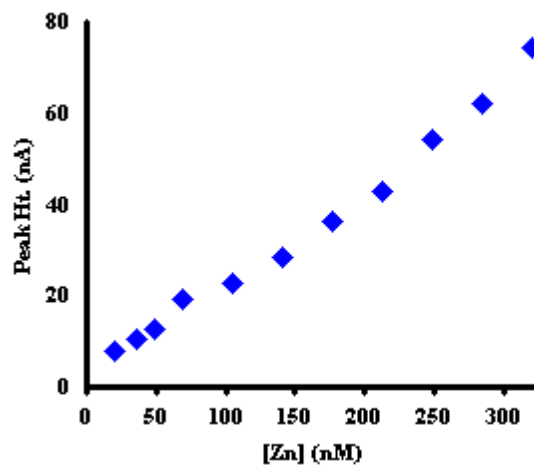


Figure 9. Relationship between TDZn and Zn-complexing ligands and river flow from bottom waters at stations 1 and 2 in the CFE during all sampling times. Both plots show no statistical significance ($p > 0.5$).

Since it is known that a large fraction of DOC in the CFE consists of humic substances (Avery et al., 2003) and that humics extracted from the CFE are very effective strong Cu chelators (Shank, 2004a; see previous section), it was important to examine the role of humics as Zn chelators. Humics isolated from Cape Fear freshwater using solid phase C₁₈ extraction were used to prepare 20 mg/L solutions (typical of the middle CFE) in UV-irradiated seawater diluted to a salinity of 10. As shown in Fig. 10a, titrations of the humic solutions did not produce an initially suppressed response on the titration curve, indicating that humics do not complex Zn or only weakly complex Zn at a level less than that detectable by the competitive ligand, APDC. It is possible that the ambient TDZn titrated out a small concentration of relative strong Zn ligands, leaving remaining Zn as either more weakly complexed Zn or inorganic Zn. Blank solutions containing diluted UV-irradiated seawater of salinity 10 containing no added humics yielded titration curves very similar to solutions containing humics (Fig. 10b). It is clear from these results that C₁₈-extractable humics are not a significant source of relatively strong Zn-complexing ligands in the CFE. In contrast to Zn, humic substances dominate the pool of strong Cu ligands in the CFE (Shank, 2004a). These results suggest that the ligands complexed to Zn and Cu are different and factors favoring strong Cu complexation in the CFE do not necessarily favor Zn complexation.



(a)



(b)

Figure 10. CLE-CSV titration curves in the (a) presence and (b) absence of C₁₈-extracted humic substances in UV-irradiated seawater of salinity ~10.

4.5. Benthic fluxes of TDZn and $\Sigma L_{i,Zn}$

As with the Cu fluxes, TDZn and Zn ligand fluxes measured using this technique were considered to be significant if the results from the linear regression of corrected concentrations vs. time produced a statistically significant fit ($p < 0.05$). When flux calculations yielded $p > 0.05$, fluxes were reported as zero. Negative values indicate a flux into sediments from overlying waters and a positive value indicates a flux out of the sediment. These experiments were performed on cores collected in November 2000, April, June, and October 2001, and March and June 2002.

Fluxes of $\Sigma L_{i,Zn}$ fluxes ranged from -1700 to 2900 nmol m⁻² d⁻¹ for both stations over the sampling period (Table 7). These values are comparable to earlier work in the lower CFE showing ligand fluxes from -980 to 1200 nmol m⁻² d⁻¹ (MacGillivray, 2003). Interestingly, the ligand flux of greatest magnitude (2900 nmol m⁻² d⁻¹) occurred at the same time as the largest TDZn flux. Statistically significant Zn ligand fluxes were observed in only 8 of the 30 cores, of which 6 were positive fluxes. This indicates sediments are generally not a significant source of Zn-complexing ligands to the water column. Measurements of Zn ligand fluxes in MacGillivray (2003) and in the present work are the only direct measurements of Zn-complexing ligand fluxes that have been reported.

Fluxes of TDZn ranged from -1100 to 1800 nmol m⁻² d⁻¹ at both sampling stations over all sampling periods (Table 8). These fluxes bracket the -56 to 300 nmol m⁻² d⁻¹ found at sta. 2 in the spring and summer in the CFE (MacGillivray, 2003). Byers (1999) observed TDZn fluxes measured using porewater profiles in the Elizabeth River (southern Virginia) ranging from -57 to 832 nmol m⁻² d⁻¹. These comparable TDZn fluxes are interesting because both the Cape Fear and Elizabeth River estuaries are subject to inputs from industrial, municipal, and commercial and naval shipping activities.

Statistically significant TDZn fluxes were observed in 12 of the 30 cores for all experiments, 9 of which were positive indicating a flux of TDZn to overlying waters from sediments. The TDZn flux of greatest magnitude (1800 nmol m⁻² d⁻¹) measured during the entire project was a positive flux occurring during November 2000 at the upper estuary site. Overall, the sporadic nature of TDZn benthic fluxes suggests that sediments are not a significant source of TDZn to waters of the CFE, similar to the conclusion of MacGillivray (2003).

Fluxes of TDZn at different estuaries are summarized in Table 9. Large, impacted estuaries such as Galveston Bay and San Diego Bay show relatively large fluxes from the sediments from 2400 nmol m⁻² d⁻¹ and 1500 to 31000 nmol m⁻² d⁻¹ respectively. The relatively small fluxes from CFE sediments probably reflect a low degree of contamination of the bottom sediments, as well as the well-flushed nature of the system.

Studies by Byers (1999) and van den Berg and Dharmvanij (1984) show estuarine sediment porewaters have relatively high concentrations of Zn-complexing ligands compared to overlying waters, suggesting that porewaters can be a source of Zn-complexing ligands. Using porewater profiles, Byers (1999) estimated Zn ligand fluxes in the Elizabeth River estuary of 144 to 1350 nmol m⁻² d⁻¹. Even though porewater gradients should be predictive of diffusive fluxes,

Season	Sampling Date	Station 1	Station 2
Fall	November 29, 2000 ($n = 2$)	2900 ($p \leq 0.02$) 0	140 ($p \leq 0.01$) 570 ($p \leq 0.001$)
	October 29, 2001 ($n = 3$)	0 1900 ($p \leq 0.01$) 2700 ($p \leq 0.001$)	0 0 0
Summer	June 26, 2001 ($n = 2$)	0 0	0 0
	June 28, 2002 ($n = 3$)	0 0 0	2000 ($p \leq 0.02$) 0 0
Spring	April 23, 2001 ($n = 2$)	0 -440 ($p \leq 0.02$)	0 0
Winter-Spring	March 7, 2002 ($n = 3$)	0 0 -1700 ($p \leq 0.01$)	0 0 0

Table 7. Fluxes of Zn-complexing ligands ($\Sigma_{L_i, Zn}$) for individual cores at stas. 1 and 2 in the CFE. Negative values indicate a flux into the sediment and positive values indicate a flux out of the sediment. Statistically significant fluxes are shown as '0'. n = number of cores per sampling site. p = significance level.

Season	Sampling Date	Station 1	Station 2
Fall	November 29, 2000 (<i>n</i> = 2)	0 1800 (<i>p</i> ≤ 0.02)	1300 (<i>p</i> ≤ 0.02) 0
	October 29, 2001 (<i>n</i> = 3)	0 0 0	0 0 -480 (<i>p</i> ≤ 0.05)
Summer	June 26, 2001 (<i>n</i> = 2)	-1100 (<i>p</i> ≤ 0.02) 0	0 0
	June 28, 2002 (<i>n</i> = 3)	460 (<i>p</i> ≤ 0.02) 520 (<i>p</i> ≤ 0.001) 0	0 1000 (<i>p</i> ≤ 0.02) 200 (<i>p</i> ≤ 0.02)
Spring	April 23, 2001 (<i>n</i> = 2)	0 200 (<i>p</i> ≤ 0.05)	0 0
Winter-Spring	March 7, 2002 (<i>n</i> = 3)	0 0 0	-160 (<i>p</i> ≤ 0.001) 1200 (<i>p</i> ≤ 0.02) 180 (<i>p</i> ≤ 0.001)

Table 8. Fluxes of TDZn in individual cores at stas. 1 and 2 in the CFE. Negative values indicate a flux into the sediment and positive values indicate a flux out of the sediment. Statistically insignificant fluxes are represented as '0'. *n* = number of cores. *p* = significance level.

Location	TDZn	Zn-L	Reference
Elizabeth River	-57 to 898	144 to 1349	Byers, 1999
Galveston Bay	2400	-	Warnken et al., 2001
San Diego Bay	1500 to 31000	-	Anderson et al., 2001
Cape Fear Estuary	-56 to 300 -1071 to 1826	-980 to 1200 -1692 to 2850	MacGillivray, 2003 This study

Table 9. Fluxes of TDZn and Zn-complexing ligands in different estuarine systems. Negative values indicate a flux into the sediment and positive values indicate a flux out of the sediment.

this approach does not account for processes occurring at the sediment water interface that may mediate exchange across the interface. Other inconsistencies may be related to disturbance of the diffusive boundary layer during sampling or as a result of removing the sediments. Thus, direct measurements of Zn ligand fluxes using the core incubation method may provide a more accurate estimate of benthic ligand fluxes than an indirect estimate using porewater profiles.

4.6. Comparison of Zn and Cu Speciation and Fluxes

Benthic fluxes of total dissolved Cu and Cu-complexing ligands were determined by Shank (2004c) using the same core incubation technique at the same two stations used during this study. Concurrent studies of Zn and Cu fluxes and speciation occurred during April 2001, June 2001, October 2001, and June 2002. No correlations were observed between benthic fluxes for TDZn and TDCu or Zn- and Cu-complexing ligands. Statistically significant TDCu fluxes occurred in 33% of the incubated cores and significant TDZn fluxes occurred in 35% of the cores. Fluxes of Cu-ligands were observed in 50% of the incubated cores and Zn-ligand fluxes were observed in 20% of the incubated cores. The majority of the cores showed neither a flux in nor out of the sediment. However, when there was a significant flux of Zn-ligands (sta. 1, October 2001) it did not coincide with a significant flux of Cu-ligands. Likewise, during June 2001 (sta. 2) and June 2002 (sta. 1), significant Cu-ligand fluxes occurred but Zn-ligand fluxes were not significant. These observations are consistent with water column measurements showing that complexation of Zn and Cu are not connected. The sediment-water exchange of Zn and Zn-complexing ligands appears to be decoupled from that of Cu and Cu-complexing ligands in the CFE, indicating that the ligands that complex the two metals are different or the conditions favoring complexation are dissimilar for the two metals.

4.7. Characterization of Zn-complexing ligands in the Cape Fear estuary

Samples containing Zn-complexing ligands typically had a titration curve (peak height vs. $[Zn]$) containing two distinguishable regions: an initially suppressed response followed by a linear response (Fig. 11a). The suppressed response was caused by complexation of added Zn by excess natural organic ligands. When the excess ligands were completely titrated the current response became linear with respect to added Zn. Titration curves were linearized by a plot of $[Zn^{2+}]/[Zn-L]$ vs. $[Zn^{2+}]$. All Ruzic-van den Berg linearizations generally yielded straight lines characteristic of a one-ligand system (Fig. 12). Scatchard plots of $[Zn-L]/[Zn']$ vs. $[Zn-L]$ for each titration curve also gave a single straight line, indicating a single ligand class. The conditional stability constants, $K'_{Zn-\Sigma L_i, Zn}$ of the Zn-ligand complexes ranged from $10^{7.0}$ to $10^{9.2}$, which is consistent with other studies where K' ranged from $10^{7.4}$ to $10^{9.4}$ (van den Berg et al., 1986, 1987; Muller and Kester, 1991; Gardner, 1999). Occasionally the Ruzic-van den Berg plot was too scattered for linearization, usually when Zn-ligand concentrations were near the detection limit. In order to obtain a Zn-ligand concentration in these cases, the linear portion of the titration curve was extrapolated back to the x-axis. In order to verify the extrapolations were valid, samples were compared by both Ruzic-van den Berg linearization and extrapolation. Both techniques gave similar Zn-ligand concentrations. When the sample contained a very small concentration of ligands or ligands that were too weak to compete with PDC, then titration curves only gave a linear response to added zinc (Fig. 11b). Zn-ligands were then reported as non-detectable. This response can be seen in Station 2 samples during April 2001 and June 2002. These observations of such small ligand concentrations was somewhat surprising given the organic-rich nature of the CFE and of the previous observations by Shank (2004a) of the large excess of strong Cu-complexing ligands in the estuary. However, these results are consistent with the assertion that Zn and Cu complexation are decoupled in the sense that different ligands must be responsible for controlling the speciation of each metal.

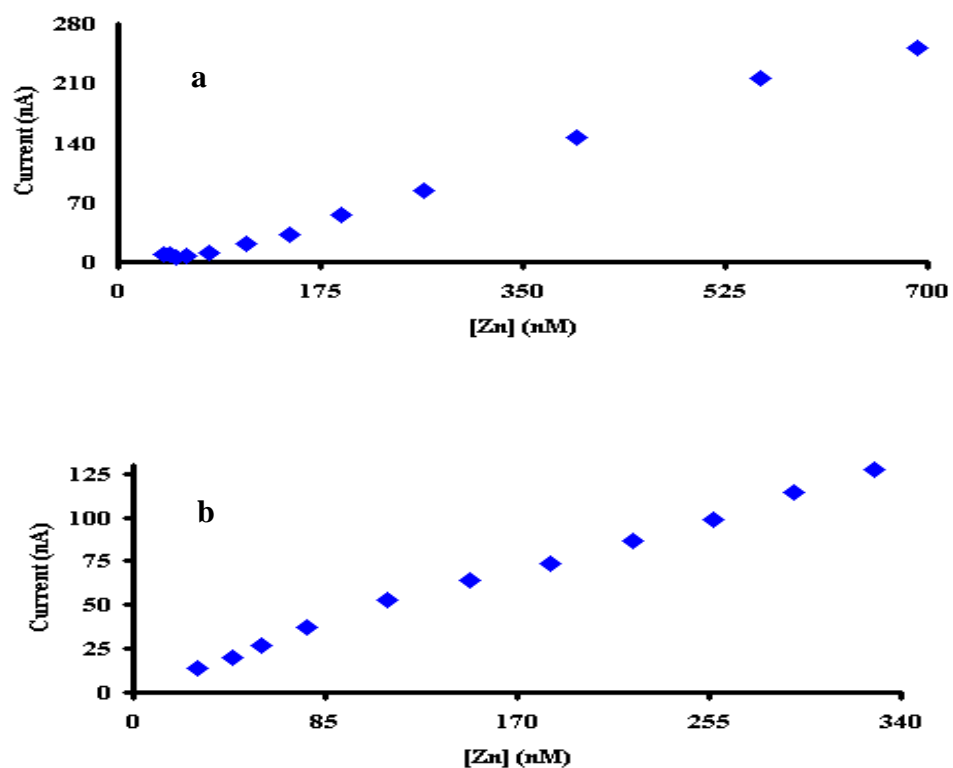


Figure 11. Typical titration curves for Zn speciation in the (a) presence and (b) absence of Zn-complexing ligands. (a) October 2001, sta. 1, timepoint T5. (b) June 2002, sta. 2, timepoint T5.

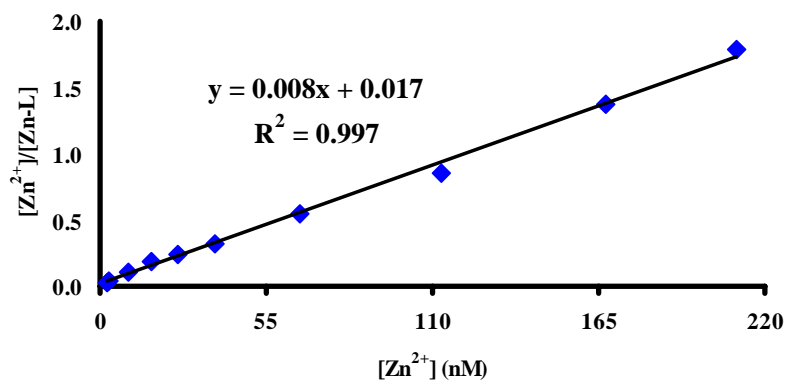


Figure 12. Ruzic-van den Berg linearization indicating the presence of one class of Zn-complexing ligands. March 2002, sta. 2, timepoint T0.

4.8. Photochemical irradiation experiments: Water column

We carried out various photochemical experiments to examine the role of sunlight in altering and degrading strong Cu-complexing ligands in estuarine water and in influencing the production or destruction of DOC and ligands in estuarine waters containing resuspended sediments. These latter experiments allow some evaluation of the effects that might be expected in estuarine and harbor environments when bottom sediments are dispersed and subjected to sunlit surface waters, as might occur during dredging and maintenance activities, shipping and berthing activities, and wind and wave events.

4.8.1. Impact of Cu on CDOM photodegradation

When 189 nM Cu(II) was added to 0.22 μm -filtered natural CFE water exposed to natural sunlight for 9.5 h, the amount of photobleaching or photochemical degradation of the colored dissolved organic matter (CDOM) was significantly less than an analogous CFE water with no added copper (Fig. 13). We hypothesize that the photoprotection occurs because the added copper act as a sink for superoxide radicals which would otherwise be available for photobleaching of CDOM. The more added copper the greater the degree of this photoprotection (Fig. 14). This hypothesis was evaluated by addition of diethylenetriaminepentaacetic acid (DTPA), which forms metal complexes which are unreactive toward superoxide (Zafiriou, 1990; Voelker et al., 2000). In the presence of DTPA, Cu affords no photoprotection of CDOM upon exposure to simulated sunlight because it no longer reacts with superoxide radicals (Fig. 14). With no copper sink present, all superoxide radicals are therefore available to photodegrade CDOM resulting in more photobleaching. Significantly more photodegradation occurs in the presence of DTPA relative to the no copper added sample because the ambient copper is also complexed in a form which does not scavenge superoxide radicals. The degree of this photoprotection afforded by ambient copper can be calculated by extrapolating the line in Fig. 15 to the y-intercept. This indicates that 6% less photobleaching of CDOM occurs in the CFE because of ambient concentration of Cu (in the form of strong Cu complexes) in the estuary.

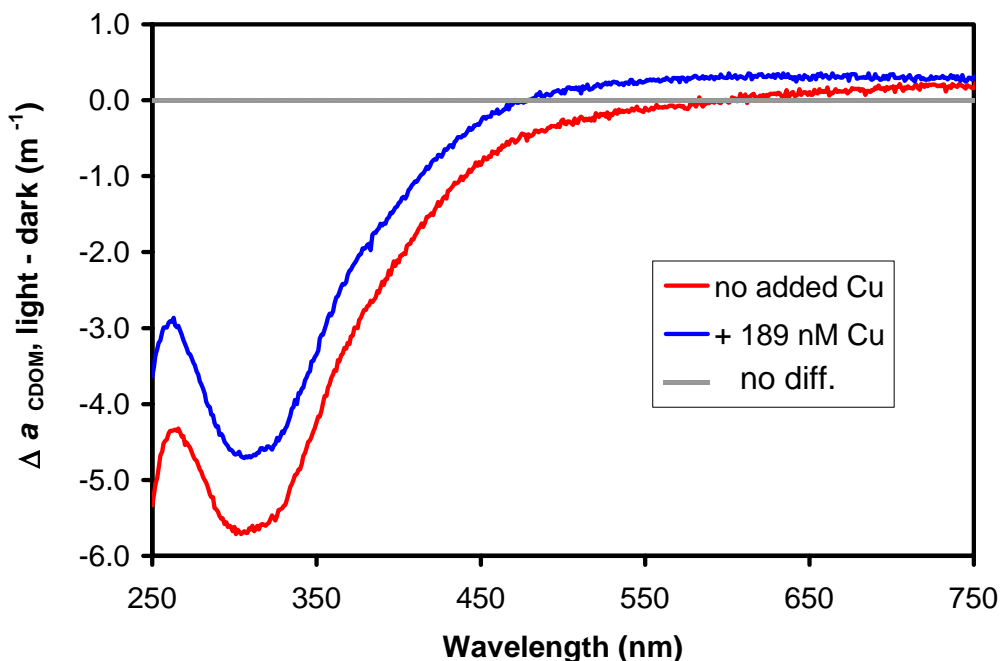


Figure 13. Change in absorbance between light-treated and dark control samples as a function of wavelength for a 0.22 μm -filtered CFE sample collected October 2000 at 1.5 m depth. Salinity ≈ 19 , $[\text{DOC}] = 744 \mu\text{M}$. Significantly lower change in absorbance occurred in the Cu-spiked sample, indicating Cu additions decreased photobleaching of CDOM in sample.

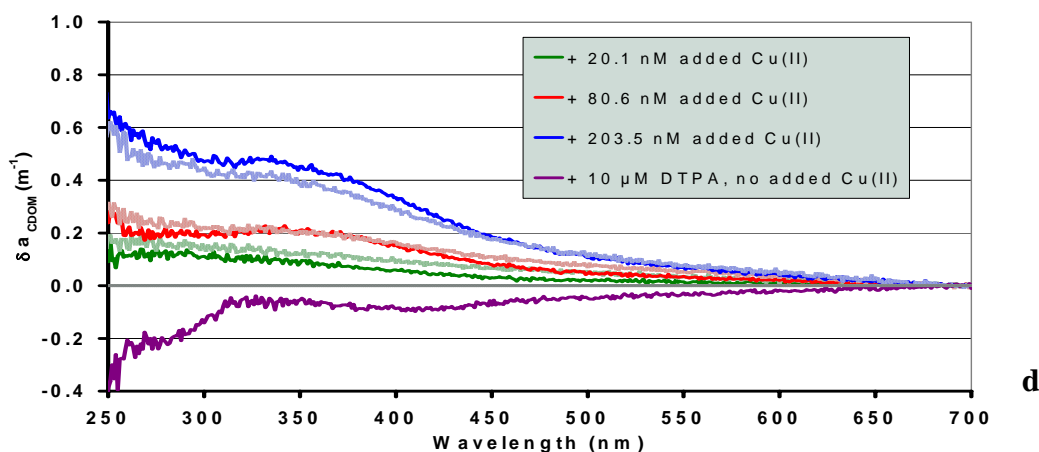


Figure 14. Increasing concentrations of Cu(II) added to CFE water increase the degree of protection against photobleaching following exposure to simulated sunlight. A strong, non-photoreactive ligand (DTPA) afforded no photoprotection.

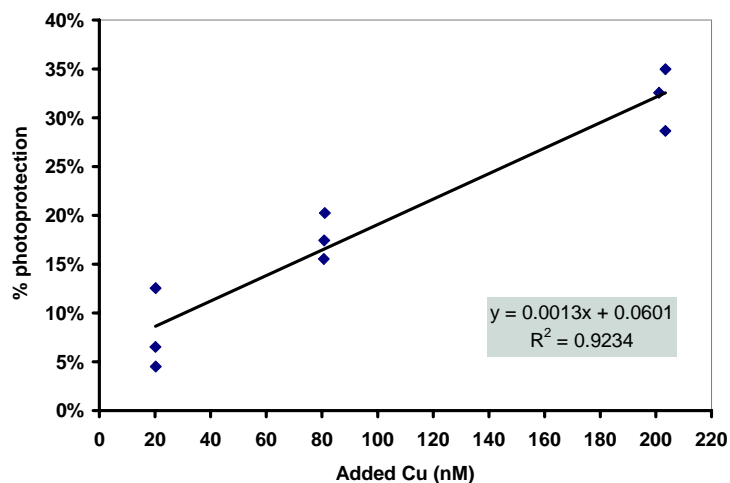
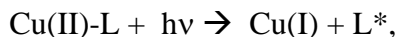


Figure 15. Linear regression of % photoprotection versus added Cu for data in Fig. 14. This analysis suggests ambient Cu resulted in 6% less photobleaching than would have occurred in the complete absence of Cu.

4.8.2. Photochemical production of Cu(I)

In a number of photolysis experiments, we have also examined the production of Cu(I), which is presumed to form as a result of a photochemically-induced ligand to metal charge transfer process involving complexed Cu(II):



where L* is oxidized ligand (Bruland et al., 1991). The results of experiments in which 0.22 μm -filtered CFE samples were exposed to simulated sunlight with subsequent formation of Cu(I) are shown in Fig. 16. In the proposed photoreduction process, it is not yet known whether or not the Cu(I) produced remains complexed, because all currently used speciation methods, including the competitive ligand electrochemical techniques used by in our work, cannot distinguish between Cu(I) and Cu(II) organic complexes (Leal and van den Berg, 1998). However, given observations in the CFE and many other systems (see section 2.2) of the large degree of organic complexation of TDCu, it seems likely that organic complexation of Cu(I) is very important also.

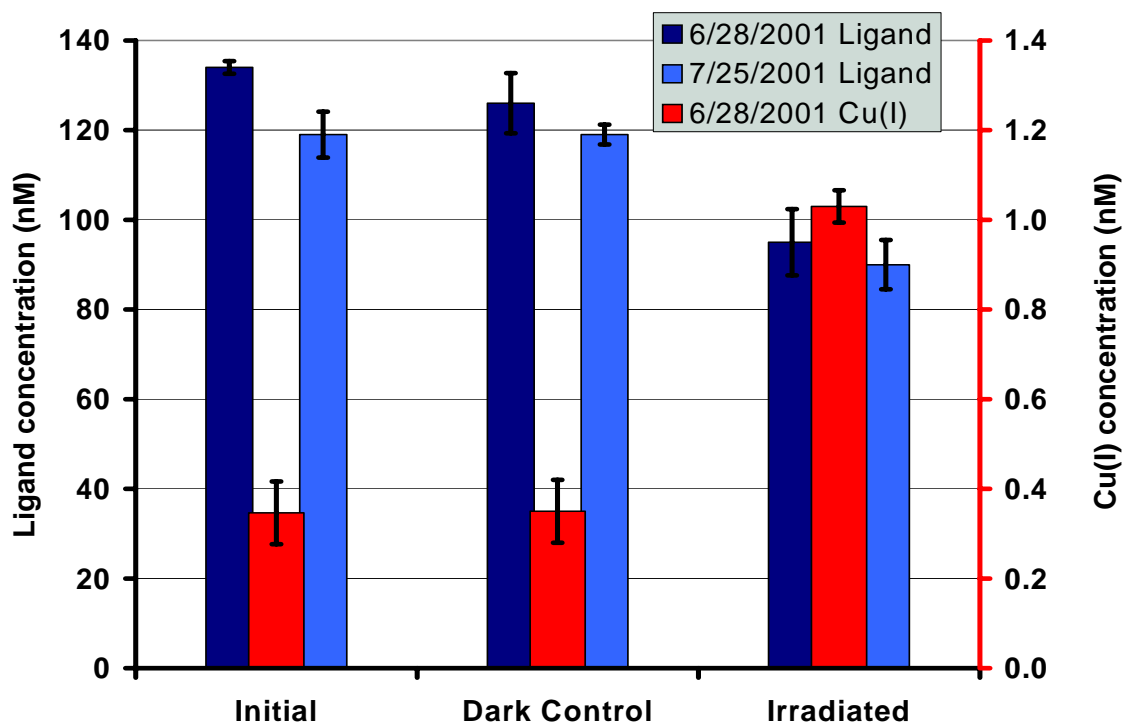


Figure 16. Concentrations of strong Cu-complexing ligands and Cu(I) in CFE samples (0.2 μm -filtered) showing a 25% loss in ligands (in two different CFE samples) and a threefold concurrent production of Cu(I) (in 6/28/01 sample) after photolysis in a solar simulator.

4.9. Photochemical degradation of strong Cu-complexing ligands

Strong Cu-complexing ligands in the CFE have been found to be extremely photoreactive. Shank (2003) conducted various degradation experiments using natural sunlight and simulated sunlight and noted significant degradation of strong Cu-complexing ligands ($\Sigma L_{i,Cu}$) in all cases. Two degradation experiments were conducted with 0.2 μm -filtered water (salinity ~ 20) exposed to simulated sunlight for 4 d (Fig. 17). One experiment was conducted under full spectrum light equivalent to the procedure in Shank (2003) whereas the second was conducted using photosynthetically active radiation (PAR) only. In natural waters, much of the UV light, while being photochemically reactive, is attenuated within a few centimeters, and even less in darkly colored waters such as in the CFE. However, PAR (400-800 nm) penetrates much more deeply. For example, in the CFE, PAR penetrates from 1.3 m in the upper estuary to 2.4 m in the lower estuary (Mallin et al., 2002).

The experiment using full spectrum showed a decrease of Cu ligand from 55 nM to 10 nM in samples exposed to light throughout the 4 d period. Dark controls also showed degradation but not to the extent of those exposed to light. Shank (2003) found ligand concentrations decreasing from 106 nM to 5 nM over the 4 days of his experiment. Samples exposed to PAR also showed ligand degradation from 50 nM to 19 nM. These results demonstrate that PAR alone is almost as effective as full spectrum light in degrading Cu-complexing ligands. This is particularly important in highly absorbing coastal waters where UV penetration is limited to very shallow depths.

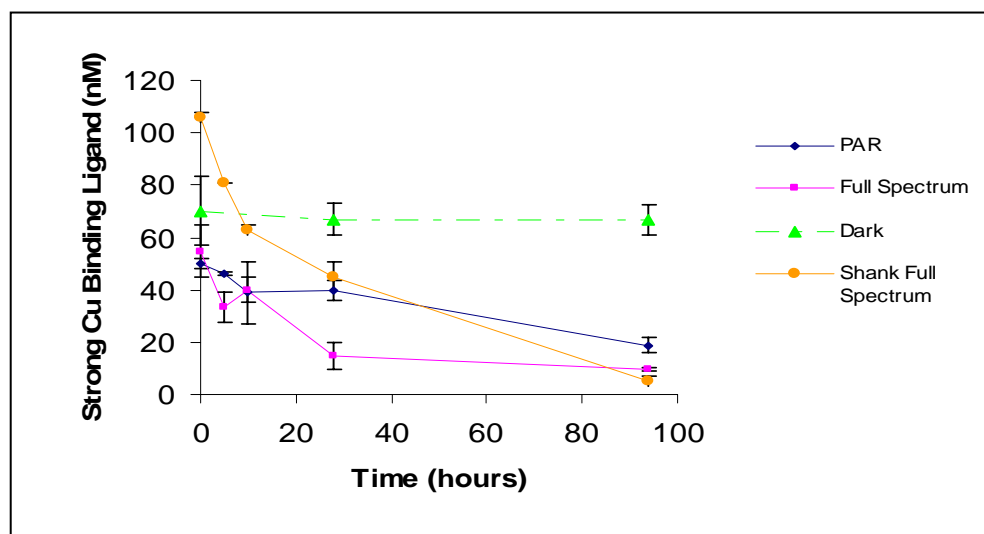


Figure 17. Photolytic degradation of strong Cu-complexing ligands in a CFE sample in solar simulator. Results are shown for full spectrum sunlight, PAR, and the dark control. Also shown are results from a previous experiment under full spectrum simulated sunlight described in Shank (2003).

4.10. Photochemical sediment resuspension experiments

The effect of sunlight on Cu exchange and speciation in resuspended estuarine sediments was examined in a M.S. thesis by Smith (2003). Relevant results from the thesis are summarized below.

A series of controlled photolysis experiments were conducted to examine possible uptake and release of dissolved Cu in filtered and unfiltered CFE water and in filtered CFE water with sediments. These experiments were conducted seasonally at sta. 1 (sandy mud) and sta. 2. (muddy sand). There was no clear trend in increase or decrease of total dissolved Cu in either sta. 1 or sta. 2 in experiments involving 0.2 μm -filtered water with no added sediment (Table 10) and 0.2 μm -filtered water with 2 g/L wet sediment added (Table 11). Most changes in both light exposed and dark flasks were relatively small and variable with respect to loss and gain of TDCu. There also did not appear to be any seasonal variability in the data. The small effect of solar irradiation in all treatments in CFE was observed even in sediments containing widely varying concentrations of leachable Cu (3-20 nmol Cu/g dry sediment) and organic carbon (0.4-16.6%) (Table 11). Slightly more consistent releases of dissolved Cu upon photolysis occurred in the set of experiments using unfiltered water (Table 12). In 6 of 8 of these trials a small release of Cu was observed, suggesting that Cu associated with suspended material in the CFE is potentially more reactive. This Cu is likely to be associated with organic matter (Davis, 1984; Herzl et al., 2003) and thus may be more easily released when a fraction of the organic matter is photolyzed. Alternatively, the Cu may be associated with Fe-Mn oxyhydroxides which may be reduced upon photolysis, leading to the release of Cu.

In addition to the relatively low-Cu containing sediments used in the photolysis experiments reported in Table 11, a second set of relatively more contaminated sediments from San Diego Bay (2300 nmol Cu/g dry sediment) were also photolyzed. Filtered (0.2 μm) water samples with sediments (2 g wet sediment per 1 L) added were resuspended; three aliquots were exposed to light and three were left in the dark. The light exposed sediment showed a very large increase in total Cu concentration relative to the dark control (Table 11). Similar photolysis experiments employing filtered CFE water and sediments collected along the salinity gradient of the CFE were conducted to include a greater range of sediment types than those presented in Table 11. In these experiments, the only significant release of Cu after photolysis occurred with sediments in the upstream estuarine station M61; the remaining stations gave small losses or gains similar to those discussed previously (Fig. 18). Sediments at M61 contain a higher leachable Cu concentration (50 nmol/g dry sediment) relative to station 1 and station 2 sediments (Table 11). Results of the photolysis experiments using the San Diego Bay and M61 sediments clearly indicate that photolytic release of Cu is only significant in sediments with relatively high Cu concentrations. The San Diego Bay sediments are known to be highly contaminated with Cu (Flegal and Sanudo-Wilhelmy, 1993) and the M61 site is adjacent to the Port of Wilmington and therefore subject to impacts from shipping activities.

Total Dissolved Cu (nM) Filtered No Sediment					
	T0	D	L	ΔD	ΔL
Station 1					
March-02	N/A				
July-02	N/A				
November-02	12.5	11.1	11.6	-1 ± 2	-0.9 ± 0.6
March-03	8.2	9.3	8.5	1 ± 0.9	0.3 ± 0.8
June-03	10.1	8.9	9.5	-1 ± 1	-0.5 ± 0.08
Station 2					
March-02	N/A				
July-02	N/A				
October-02	N/A				
April-03	27.2	13.7	11.4	-13 ± 3	-16 ± 0.6
June-03	8.3	10.3	8.9	2 ± 0.04	0.6 ± 0.8

Table 10. Results of resuspension experiments in the absence and presence of simulated sunlight in samples from the CFE. Triplicate water samples were 0.2 μm -filtered with no added sediments. T0, D, and L indicate average TDCu concentrations initially, in the dark control, and after 9 h irradiation, respectively. Change (D) in D and L are changes in dark and light concentrations, respectively, compared to T0 samples. Not applicable (N/A) indicates results not obtained.

Total Dissolved Cu (nM/g dry sediment)							
Filtered + Sediment							
	T0	D	L	ΔD	ΔL	nmol Cu/g dry sed	%OC
Station 1							
March-02	6.7	6.4	7.0	-0.4 ± 1	0.5 ± 2	N/A	1.5
July-02	7.7	8.5	8.6	1 ± 2	2 ± 4	N/A	3.5
November-02	19	10.9	13.4	-20 ± 1	-13 ± 6	20 ± 2	5.7
March-03	8.3	6.6	6.1	-6 ± 2	-1 ± 1	8 ± 0.4	4.9
June-03	6.3	8.2	7.3	5 ± 4	1 ± 0.4	10 ± 4	6.9
Station 2							
March-02	4.1	5.1	5.5	1 ± 1	2 ± 0.4	N/A	2.6
July-02	6.5	7.4	5.8	1 ± 5	-1 ± 2	N/A	0.43
October-02	5.0	5.2	11.6	0.4 ± 9	11 ± 4	3 ± 0.4	3.9
April-03	10.0	6.9	9.3	-3 ± 4	-4 ± 3	7 ± 1	4.5
June-03	9.0	7.8	10.6	-7 ± 4	2 ± 3	5 ± 0.1	16.6
San Diego Bay							
April-03	20.8	31.8	46.6	22 ± 5	47 ± 6	2271 ± 353	5.2

Table 11. Results of resuspension experiments in the presence and absence of simulated sunlight in samples from the CFE and San Diego Bay. Triplicate water samples were 0.2 μm -filtered with added sediment (2 g wet sediment per L). T0, D, and L indicate average TDCu concentrations initially, in dark controls, and after 9 h irradiation, respectively. Change (D) in dark and light concentrations, respectively, are compared to T0 samples. Also shown are leachable (1 M HCl) Cu concentrations and percent organic carbon in sediments. Not applicable (N/A) refers to results not obtained.

Total Dissolved Cu (nM) Unfiltered No Sediment					
	T0	D	L	ΔD	ΔL
Station 1					
March-02	N/A				
July-02	7.4	6.5	8.1	-0.9 ± 0	0.7 ± 2
November-02	0.92	1.5	2.5	1 ± 2	-0.5 ± 2
March-03	12.6	7.9	9.9	-5 ± 1	-3 ± 1
June-03	6.1	9.2	10.0	3 ± 0.2	4 ± 0.9
Station 2					
March-02	N/A				
July-02	3.4	4.4	6.3	1 ± 0	3 ± 1
October-02	5.7	6.6	11.6	-0.4 ± 1	3 ± 0.9
April-03	10.8	10.5	12.0	-0.3 ± 0.7	1 ± 0.09
June-03	8.7	10.3	10.1	2 ± 0.3	1 ± 0.3

Table 12. Results of resuspension experiments in the presence and absence of simulated sunlight in samples from the CFE. Triplicate water samples were unfiltered with no added sediments. T0, D, and L indicate average TDCu concentrations initially, in the dark controls, and after 9 h irradiation, respectively. Change (D) in D and L are changes in dark and light concentrations, respectively, compared to T0 samples. Not applicable (N/A) refers to results not obtained.

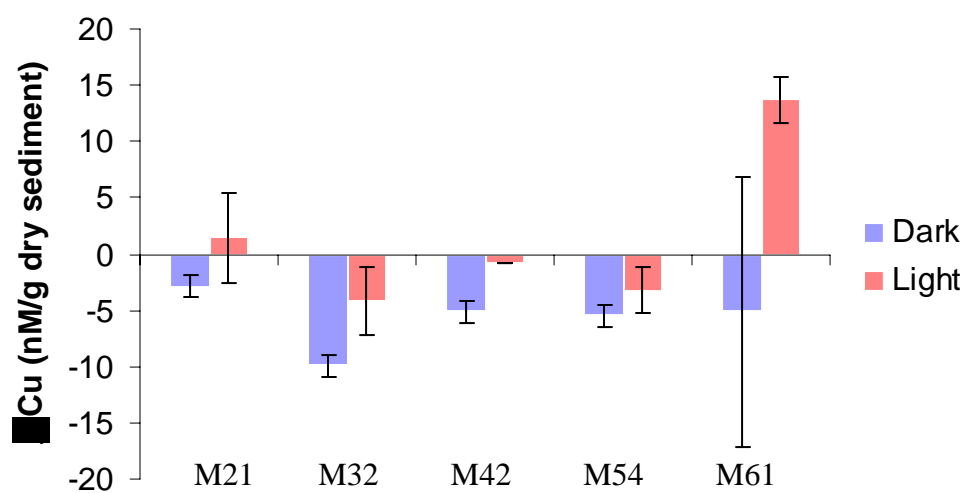


Figure 18. Change (Δ) from initial values to after 9 h of Cu (nM per g dry sediment) for triplicate light-exposed and dark controls of 0.2 μm -filtered CFE water with added sediment (2 g wet sediment/L) at stations along the salinity gradient in the CFE.

In the CFE transect, percent organic carbon was highly correlated to the amount of Cu in the sediments (Fig. 19). This relationship is similar to that found by Turner et al. (2002), who also found acid-leachable Cu to be positively correlated to particulate organic carbon in sediments from the Mersey estuary, UK. Previous studies have also found that the amount of Cu found in riverine, estuarine and coastal sediments is associated with organic material (Gerringa, 1990; Shaw et al., 1990; Shulkin and Bogdanova, 2003). Although the total Cu concentration at M61 increased significantly after photolysis of resuspended sediments (Fig. 18), other sediments with relatively low Cu concentrations showed little or no change upon exposure to light even though their percent organic carbon consistently increased toward M61. Dark controls all decreased suggesting a net uptake of Cu by these sediments along this gradient. Herzl et al. (2001) found similar results in the Tamar estuary in the United Kingdom for dark sediment resuspensions. In that work, after five hours of resuspension, total dissolved Cu concentrations decreased by 50% due to adsorption of Cu onto particles (Herzl et al., 2001). The present study found that after 9 h, total Cu concentrations decreased by an average of 41%. The release at M61 should be viewed therefore as a minimum production value as some Cu may also have been taken up by the sediment. In contrast, Shulkin and Bogdanova (2003) found that riverine sediments that contained elevated amounts of Cu and organic matter resulted in greater release of dissolved Cu in resuspension experiments.

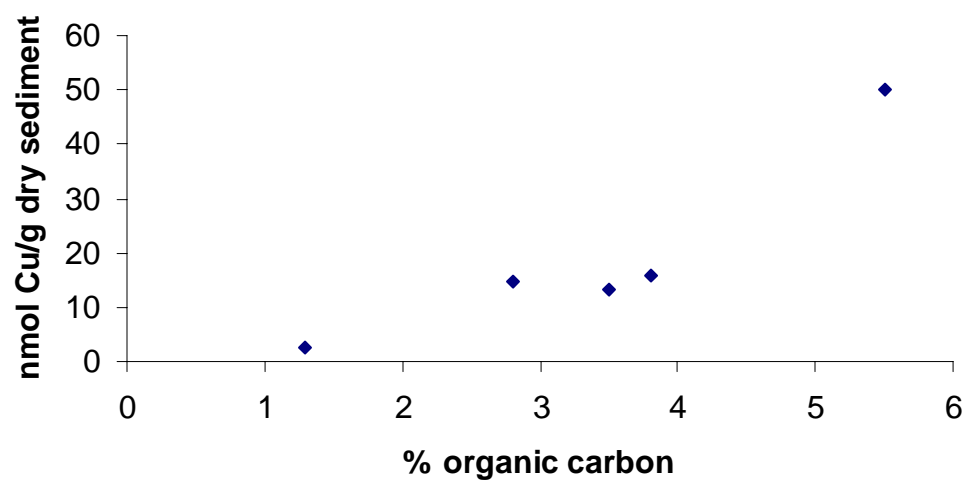
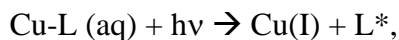


Figure 19. Percent organic carbon from triplicate dry sediment samples vs. Cu (nmol per g dry sediment) for sampling sites M23, M35, M42, M54, and M61, respectively, in the CFE in April 2003.

4.11. Cu redox behavior

Copper (I) extractions were completed on samples collected for the three types of photochemical experiments to determine the effect of sediment resuspension and photolysis on Cu redox speciation. Samples that were 0.2 μm -filtered with no sediment added had large increases in Cu(I) concentrations in all cases when exposed to simulated sunlight (Fig. 20). Dark samples increased as well in most cases although to a lesser extent than irradiated samples. This suggests that Cu(I) is produced photochemically in CFE water:



where L is some strong Cu-complexing ligand and L* is oxidized ligand (Bruland et al., 1991).

Earlier studies have suggested that Cu(I) can be reduced from Cu(II) photochemically or biologically (Moffett and Zika, 1988). Copper (II) may also be reduced in redox reactive complexes by photochemical means (Moffett and Zika, 1988). Microbially-mediated reduction of Cu(II) could explain the increase in dark samples. Various compounds such as thiols are also known to reduce Cu(II) to Cu(I) (Leal and van den Berg, 1998). Thiols have been detected in sediment porewaters (Shea and MacCrehan, 1998) and estuarine waters (Tang et al., 2000), and may explain how Cu(I) may be formed non-photochemically.

Unfiltered samples with no sediments were also irradiated in order to determine the impact of ambient particles on Cu(I) photoproduction. Light exposed samples had an increase in Cu(I) compared to initial values (Fig. 21). Dark samples also showed an increase but never as great as in the light. The magnitude of the increase in light exposed samples was always equal to or less than in filtered samples. In some cases (11/02) there was a significant decrease in Cu(I) compared to filtered samples. These results suggest that ambient particles either have no impact on Cu(I) production or cause a net uptake/oxidation of Cu(I). Kieber et al. (1992) performed a similar photolysis study on chromium, using filtered and unfiltered water exposed to dark and sunlight for one hour. In samples that were filtered, there was no apparent change in chromium concentration comparing before and after photolysis. In samples that were left unfiltered, chromium was reduced during photolysis indicating that particles play a role in chromium speciation during light exposure (Kieber et al., 1992). These results indicate that different transition metals are controlled by unique redox speciation, and there must be another component that effects Cu(I) concentrations in the CFE.

Filtered water samples with 2 g wet sediment per L were also irradiated in order to evaluate the role of sediments in the redox speciation of Cu (Fig. 22). Samples with added sediments showed increases in light exposed samples. As was the case with unfiltered samples, increases in the light were equal to or smaller than in filtered water suggesting that sediments were not a significant source of Cu(I). In five of the seven dark samples there were significant decreases in Cu(I). This indicated that sediments, as was suggested for ambient particles, may be a net sink of Cu(I) in estuarine waters either through absorption or increased oxidation.

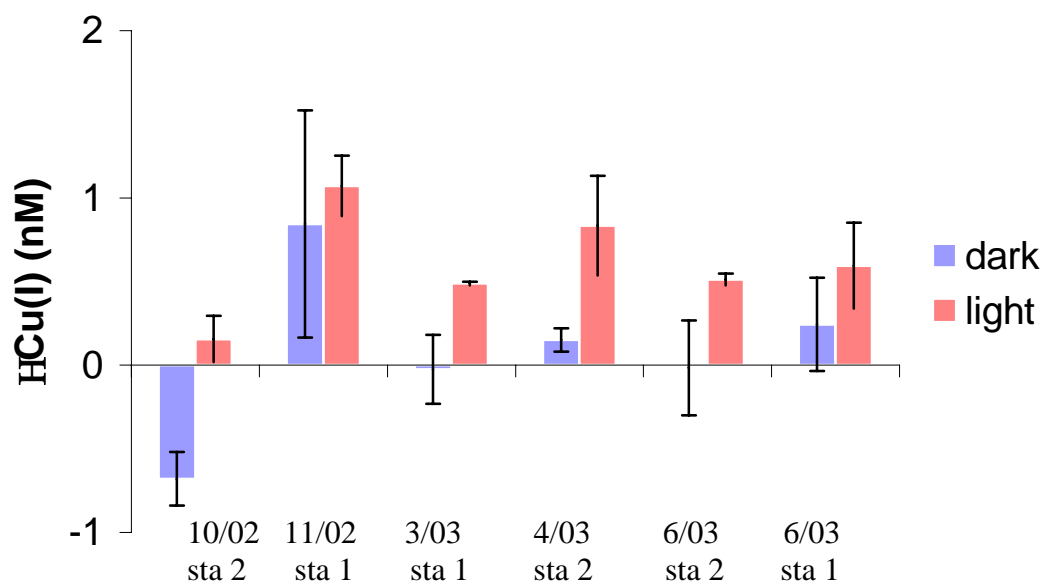


Figure 20. Change (Δ) in Cu(I) concentration (nM) for dark controls and irradiated samples after 9 h compared to initial values, for 0.2 μm -filtered CFE water in triplicate samples from two sites.

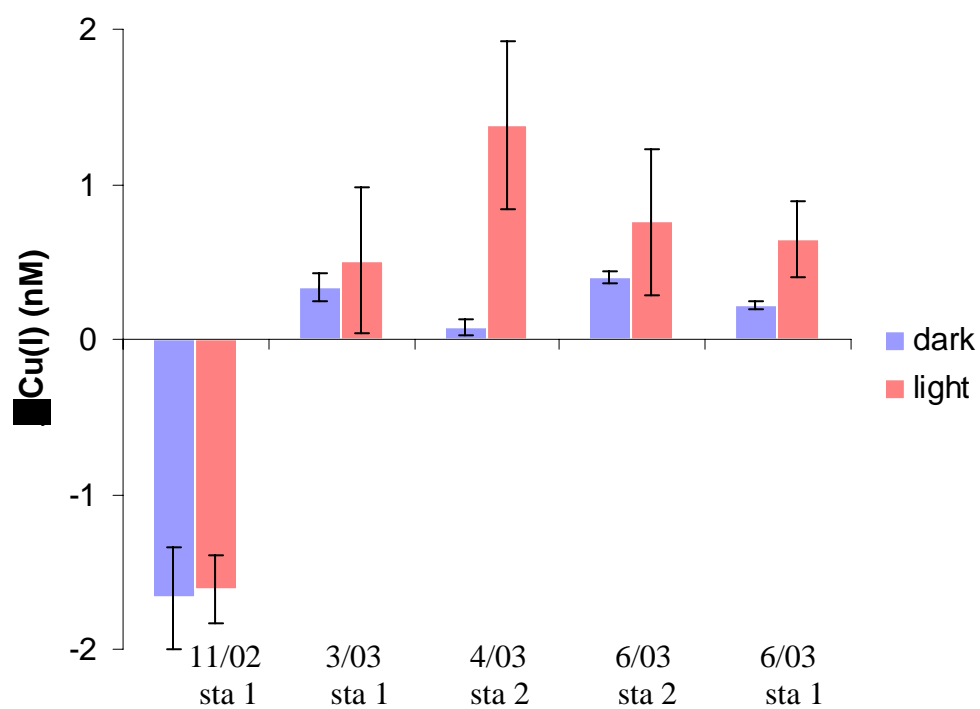


Figure 21. Change (Δ) in Cu(I) concentration (nM) for dark controls and irradiated samples after 9 h compared to initial values, for unfiltered CFE water in triplicate samples from two sites.

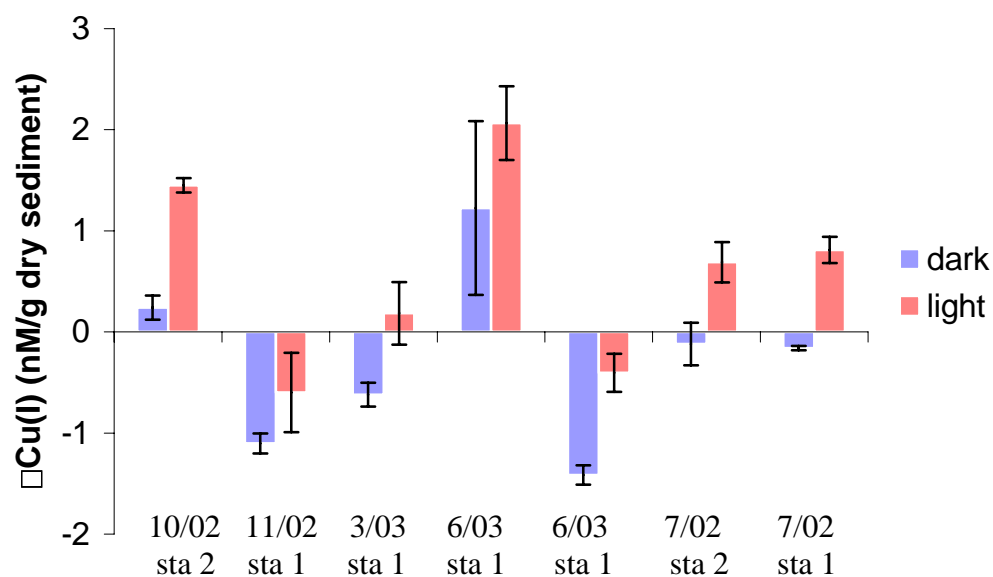


Figure 22. Change (Δ) in Cu(I) concentration (nM/g dry sediment) for dark controls and irradiated samples after 9 hours compared to initial values, for 0.2 μ m-filtered CFE water triplicate samples with added sediments (2 g wet sediment per L) from two sites.

4.12. Collaborative studies with SERDP projects CP-1156 and CP-1158

Several collaborative studies were carried out between our research group (CP-1157) and research groups from SPAWAR-San Diego (CP-1156) and the University of Wisconsin at Madison (CP-1158). These collaborations included an interlaboratory comparison study for total dissolved Cu and Zn and metal speciation involving all three groups; collaborative sampling and ultrafiltration studies of the Cape Fear estuary involving CP-1157 and CP-1158; and benthic flux studies in San Diego Bay involving CP-1156 and CP-1157. Results from these collaborations are described below.

4.12.1. Interlaboratory comparison of total dissolved metals and metal speciation

In response to SERDP's request, groups CP-1156, CP-1157, and CP-1158 participated in an intercalibration exercise using cleanly collected samples from San Diego Bay (collected and distributed by CP-1156) and the Cape Fear Estuary (collected and distributed by CP-1157). Our group collected a 50 L sample on 17 July 2000 in the lower Cape Fear estuary (salinity ~23) from 1-1.5 m depth by peristaltic pump, using acid-washed FEP-lined PVC tubing (Nalgene), sequential in-line acid-washed 1.2 and 0.22 μm polypropylene capsule filters (MSI Calyx), and acid-washed C-Flex tubing (Cole-Parmer) connections through the pump head. The bulk sample was collected in an acid-washed 50 L Nalgene polyethylene carboy that was covered with an opaque black plastic bag. Tubing intake was attached to a PVC-encased weight lowered on nylon rope, deployed from a 2 m long PVC-encased boom upwind of our fiberglass boat. This sample was stored in dark at 4° C. The carboy was shaken before aliquoting samples to CP-1156 and CP-1158 (in bottles they provided) in a clean room on 18 July 2000. Similarly we received a 0.22 μm -filtered sample collected from San Diego Bay by CP-1156 on 24 July 2000. These samples were subsequently analyzed for concentrations of total dissolved Cu (TDCu) and Cu speciation using methods normally used by each research group. A research group led by Dr. John R. Donat of Old Dominion University also participated in the intercomparison as a matter of professional interest; their results are tabulated with those of the SERDP groups in Tables 13 and 14 below.

The results for TDCu were statistically indistinguishable between groups, each of which used different analytical techniques (Table 13). Results for Cu speciation (Table 14) were internally consistent, but were more difficult to interpret due to the fact that the different research groups used different analytical techniques to determine concentrations of Cu-complexing ligands and conditional stability constants of the CuL_x complexes (where L_x is the ligand). Internal consistency was demonstrated by the fact that the ligand concentrations detected by the various methods decreased in a generally linear fashion as the competition level of the detection method increased (Fig. 23). The competition level is described by the logarithm of the quantity $\alpha = [\text{CuAL}] / [\text{Cu}^{2+}]$, where AL is the added ligand (e.g., 8-hydroxyquinoline, salicylaldoxime, etc.), CuAL is the Cu-added ligand complex, and Cu^{2+} is free hydrated cupric ion. A highly competitive ligand such as 8-hydroxyquinoline pulls more Cu away from the natural Cu-ligand complexes in a sample, leaving a smaller fraction of very strong natural Cu-ligand complexes to be detected. Conversely, a method such as anodic stripping voltammetry (ASV) gives a relatively low level of competition (i.e., a smaller $\log \alpha$); hence a larger concentration of natural

Cu-complexing ligands is detected (Bruland et al., 2000). These trends are consistent with the view that natural Cu-complexing ligands form a continuum of strengths in natural waters. It is important to note that determinations of free cupric ion activity, as measured by pCu (the negative log of the Cu^{2+} activity), gave very similar results (e.g., 12.7-14.3 for the Cape Fear) for the CSV techniques regardless of the competing ligand used (Table 14). This is significant because it is the free cupric ion activity which is most important in evaluating potential toxicity and bioavailability in natural waters.

The first intercalibration exercise did not address whether or not the different research groups can obtain the same results when using the exact same analytical techniques. To address this shortcoming, an intercalibration exercise using San Diego Bay water was performed in January 2001 with the research groups using identical techniques. Results from the second intercalibration show that results obtained for TDCu were statistically indistinguishable between groups. Strong Cu-complexing ligand concentrations, determined using the CLE-CSV technique with 8-HQ as competing ligand at the approximately same level of competition ($\log \alpha_{\text{CuAL}} = 4.8-5$), were also statistically similar among groups (Table 15).

Total dissolved Zn concentrations also agreed well in the second exercise (Table 16) although Zn speciation could not be compared because the other groups did not routinely perform such analyses in their projects.

Group	San Diego [Cu] (nM)	Cape Fear [Cu] (nM)
SPAWAR (CP-1156)	30.2 ± 0.3 (n = 3)	9.9 ± 0.5 (n = 3)
UNCW (CP-1157)	24.3 ± 2.7 (n = 4)	8.1 ± 1.5 (n = 6)
UWM (CP-1158)	24.8 ± 1.4 (n = 2)	7.0 ± 0.5 (n = 3)
ODU	32.0 ± 5.4 (n = 2)	10.2 ± 2.5 (n = 3)
Result of Model II ANOVA (95% C.L.)	No differences	No differences

Table 13. Results of first interlaboratory comparison for TDCu using a sample collected in July 2000 from the CFE. Uncertainties are ± 1 standard deviation. Abbreviations: n = number of analyses; UWM = University of Wisconsin at Madison (CP 1158); UNCW = University of North Carolina at Wilmington (CP 1157); SSC-SD = SPAWAR Systems Center at San Diego (CP 1156); ODU = Old Dominion University. Methods used were graphite furnace atomic absorption spectrometry after preconcentration using APDC/DDDC (SPAWAR); cathodic stripping voltammetry using 8-hydroxyquinoline or tropolone (UNCW); inductively-coupled plasma mass spectrometry after online preconcentration using immobilized 8-hydroxyquinoline (UWM); cathodic stripping voltammetry using salicylaldoxime (ODU).

Group	Method	Cape Fear Estuary				San Diego Bay			
		[L] nM	pCu	log K'	log α	[L] nM	pCu	log K'	log α
UWM	CSV-8HQ	63	14.1	13.2	>6	15	12.3	11.8	>4
UNCW	CSV-8HQ	112	14.3	13.6	5.1	15	n.c.	13.5	4.8
ODU	CSV-SA	137	12.7	12.1	4.2	18	10.8	12.9	4.2
SPAWAR	Orion ISE	141	11.0	n.c.	~3	81	11.1	n.c.	~3
UWM	ASV	----Organic interferences----				77	n.c.	9.5	2.4
ODU	ASV					77	n.c.	10.3	~2

Table 14. Results of first interlaboratory comparison for dissolved Cu speciation using samples collected in July 2000 from the Cape Fear estuary and San Diego Bay. Uncertainties are ± 1 standard deviation. These results were compiled by Dr. M. Shafer of the University of Wisconsin at Madison (CP-1158). Abbreviations as in Table 13. Method abbreviations: CSV-8HQ = cathodic stripping voltammetry using 8-hydroxyquinoline as the competing ligand; CSV-SA = cathodic stripping voltammetry using salicylaldoxime as the competing ligand; Orion ISE = Ion selective electrode (Orion jalpaite – 94-29); ASV = anodic stripping voltammetry.

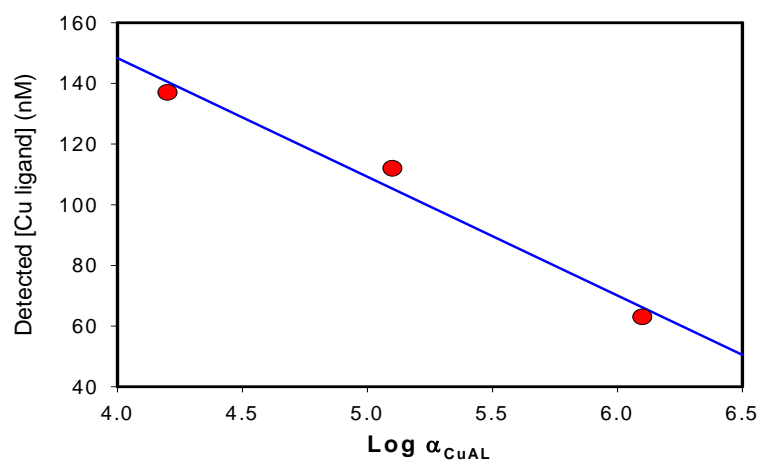


Figure 23. Plot of detected Cu-complexing ligand concentrations versus $\log \alpha_{\text{CuAL}}$. The latter quantity expresses the competition level exerted in the speciation determination experiment, with a larger $\log \alpha_{\text{CuAL}}$ expressing the ability of the experiment to detect stronger classes of ligands.

Group	Method	[Ligand] nM	TDCu (nM)	log K'	log α
UWM	CSV-8HQ	5.5 \pm 0.7	9.3 \pm 0.4	13.3 \pm 0.9	4.8
UNCW	CSV-8HQ	4.6 \pm 0.4	7.6 \pm 0.9	14.1	5.0
SPAWAR	CSV-8HQ	n.a.	9.7 \pm 0.2	n.a.	n.a.
		No diff. (t-test, 95% CL)	No diff. (mod. II ANOVA, 95% CL)		
UWM	ASV	62 \pm 7		9.6	

Table 15. Results of second interlaboratory comparison for total dissolved Cu and Cu speciation using a sample collected in January 2001 from San Diego Bay (station 3). Uncertainties are ± 1 standard deviation. Abbreviations as in Tables 13 and 14.

Group	TDZn (nM)
SPAWAR	199 \pm 5
UNCW	166 \pm 19
UWM	174 \pm 5

Table 16. Results of second interlaboratory comparison for total dissolved Zn using a sample collected January 2001 in San Diego Bay (station 19). Uncertainties are ± 1 standard deviation. Methods used were graphite furnace atomic absorption spectrometry after preconcentration using APDC/DDDC (SPAWAR); cathodic stripping voltammetry using APDC (UNCW); inductively-coupled plasma mass spectrometry after online preconcentration using immobilized 8-hydroxyquinoline (UWM). Institution abbreviations as in Tables 13-15.

4.12.2. Collaborative sampling and ultrafiltration studies of the Cape Fear estuary

In October 2000, Dr. Martin Shafer and members of his research group at the University of Wisconsin-Madison (CP-1158) sampled several stations in the Cape Fear Estuary and subsequently fractionated several of these samples using tangential flow ultrafiltration in our laboratory. Splits of various ultrafiltration fractions were given to us for analysis of total dissolved Cu, Cu speciation, and DOC. Analytical results from these experiments are shown in Tables 17 and 18.

Two interesting points are evident from these data. First, most of the DOC in the organic-rich Cape Fear Estuary is in size classes associated with “truly dissolved” or small colloids (i.e., ≤ 10 kDa). These measurements are consistent with many previous observations in this estuary indicating that DOC is largely conservative during estuarine mixing; that is, it is not appreciably added or removed over the salinity gradient. Larger colloidal material would be expected to coagulate in the low salinity zones of the estuary. This is consistent with our hypothesis that most DOC in the estuary is relatively refractory material, decomposed to a large extent from higher molecular weight (larger colloidal or particulate) source materials. Copper speciation measurements indicate that most of the strong Cu-complexing ligands in the estuary derive from this small colloidal size fraction. This observation contrasts with previous work by Wells et al., (1998) in Narragansett Bay, Rhode Island, in which it was found that most Cu-complexing ligands were found in the “truly dissolved” size fraction (<1 kDa) and less abundantly in the colloidal fraction (1-7 kDa). However, differences of this nature are not unexpected, in that the Cape Fear estuary is much more organic-rich than the Narragansett and has very different physical and chemical characteristics.

A second ultrafiltration experiment was performed on Cape Fear estuary samples by Dr. M. Shafer’s group (CP-1158) in April 2002 and analyzed by our group for DOC, TDCu, and Cu speciation. Results from these experiments are fully discussed in Shank (2003) and Shank et al. (2004a). Significance of the results to understanding Cu speciation in the CFE have been summarized previously in this report (see section 4.2).

Dissolved organic carbon (μM)					
	0.4 μm filtered	1 kDa permeate	1 kDa retentate	10 kDa permeate	10 kDa retentate
Upper	1616	431	1206	1262	-----
Middle	958	243	630	738	251
Lower	334	123	221	295	59

Size distribution of dissolved organic carbon				
	Mass Balance	< 1 kDa	1 kDa - 10 kDa	> 10 kDa
Upper	100%	27%	51%	22%
Middle	103%	25%	52%	26%
Lower	106%	37%	51%	18%

Table 17. Results of dissolved organic carbon analyses in an ultrafiltration experiment at three sites in the Cape Fear estuary, October 2000. Values for 10 kDa retentate were computed by subtracting concentrations in 10 kDa permeate from those in the 0.4 μm filtrate.

Lower Estuary				
	0.4 μm filtrate	1 kDa permeate	10 kDa permeate	10 kDa retentate
TDCu (nM)	2.82 ± 0.36	0.86 ± 0.17	2.65 ± 0.08	0.48 ± 0.04
[L₁] (nM)	71.7 ± 7.6	2.9 ± 0.5	71.0 ± 6.0	0.7 *

Middle Estuary				
	0.4 μm filtrate	1 kDa permeate	10 kDa permeate	10 kDa retentate
TDCu (nM)	6.92 ± 0.30	1.40 ± 0.17	4.95 ± 0.37	1.96 ± 0.21
[L₁] (nM)	76.6 ± 0.4	6.3 ± 0.1	67.2 ± 8.3	9.4 *

Table 18. Results of analyses of total dissolved Cu (TDCu) and strong Cu-complexing ligands ([L₁]) in an ultrafiltration experiment at two sites in the Cape Fear estuary, October 2000. Values for 10 kDa retentate were computed by subtracting concentrations in 10 kDa permeate from those in the 0.4 μm filtrate.

4.12.3. Benthic flux studies in San Diego Bay

Collaborative sampling (involving CP-1156 and CP-1157) for benthic fluxes of TDCu and Cu-complexing ligands took place in March 2003 in San Diego Bay. The site chosen was station 27 at the head of the bay; this site contains predominantly muddy sediments. See Chadwick et al. (2004) for additional details about Cu behavior in San Diego Bay. Cores were obtained by divers and a vessel supplied by SPAWAR and incubated in the SPAWAR laboratories using previously described methods for flux determinations. Results from this study are shown in Table 19.

A statistically significant flux of strong Cu-complexing ligands ($\Sigma L_{i,Cu}$) occurred in only one of the three cores studied; this flux was relatively small and directed into the sediment. Statistically significant fluxes of TDCu occurred in two of three cores. These fluxes were of modest magnitude, with one flux directed into the sediment and one flux out of the sediment.

An initial goal of this collaborative work was to perform an interlaboratory comparison of flux determinations using both the core incubation technique employed by our group (CP-1157) and core incubations and benthic chambers employed by the SPAWAR group (CP-1156). The SPAWAR group collected TDCu and ($\Sigma L_{i,Cu}$) data from three incubated cores and a benthic chamber. Although benthic fluxes were not specifically calculated by the group, an examination of their data show very modest fluxes of TDCu into the sediment from the benthic chamber and one of three incubated cores, and modest fluxes out of the sediment from two of three incubated cores (I. Rivera-Duarte, 2004, pers. comm.). These small values are comparable to those obtained by UNCW (CP-1157) and fall well within the range of TDCu fluxes previously determined in San Diego Bay (-1600 to 4700 nmol m⁻² d⁻¹; Chadwick et al., 1999).

Measurements of Cu complexation capacity (obtained by Cu titrations and detection using the Cu ion-selective electrode (Cu-ISE) suggest uptake of ligands by sediment in the core incubations (I. Rivera-Duarte, 2004, pers. comm.). However, as is evident from the results of the Cu speciation interlaboratory comparison (see section 4.12.1), the Cu-ISE speciation method detects considerably broader classes of ligands, including much weaker ligands, than does the CLE-CSV method used by our group (CP-1157). Therefore, the speciation results are not directly comparable, although the trend of ligand uptake by sediments at sta. 27 in San Diego Bay was found by both methods. A more valid intercomparison for benthic flux methods must include a broader range of methods for determining speciation, with at least some individual methods having the same detection window (i.e., α_{MAL}). For example, a study of the type used by the SERDP collaborators (CP-1156, CP-1157, and CP-1158) and by Bruland et al. (2000) for comparing Cu speciation methods in estuarine water column samples, should be performed for a more valid comparison.

Core	Ligand flux (nmol m ⁻² d ⁻¹)	TDCu flux (nmol m ⁻² d ⁻¹)
A	0	328
B	0	0
C	-165	-153

Table 19. Benthic fluxes of strong Cu-complexing ligands ($\Sigma L_{i,Cu}$) and TDCu from cores collected in March 2003 from station 27 located at the head of San Diego Bay. Negative values indicate a flux into the sediment; positive values indicate a flux into the sediment.

4.13. Response to action item:

Determine the impact on copper concentrations of light extinction with depth and diurnally and discuss how this will affect when free copper should be measured during a sampling day. Explain whether diurnal/sunlight effects will be included in the protocol for water body sampling/characterization and hence, modeling of same. Explain the effects of dredging on the fate and transport of copper and zinc to the experimental design.

4.13.1. Sunlight effects and implications

The role of photochemically-induced changes on Cu speciation and Cu-organic carbon-particle interconversions is dependent on the intensity and efficiency of light energy regions of the light spectrum encompassing ultraviolet (UV; 200-400 nm) and photosynthetically available radiation (PAR; 400-700 nm). These are in turn dependent on the optical properties of the water column (e.g., concentrations of particulate matter and colored dissolved organic matter) which control the penetration of UV and PAR. In organic-rich, darkly colored waters such as those of the CFE, UV penetration is on the order of a few centimeters whereas PAR may penetrate 1-2.5 m (R.F. Whitehead, unpublished data). In the open ocean, UV penetration (310 nm) may extend to a few tens of meters in oligotrophic open ocean waters to a few meters in coastal or eutrophic waters (Whitehead et al., 2000), whereas PAR may extend to 100 m or more in clear open ocean waters.

Although we have shown that photochemical degradation of Cu-complexing ligands under simulated solar light can be an effective sink for ligands, this process acts over the long-term and probably has little effect on ligand concentrations on short-term (e.g., diurnal) time scales in relatively organic-rich harbors and estuaries. Our experimental results on the role of light in affecting Cu(I)-Cu(II) transformations, however, suggest that changes in redox speciation of Cu do occur on diurnal time scales in estuarine waters. We have shown that simulated sunlight does cause some photoreduction of Cu(II) to Cu(I) (where both redox states exist as organically complexed forms). Existing CLE-CSV techniques for Cu speciation can not distinguish between organically complexed Cu(I) and Cu(II) (Leal and van den Berg, 1998); therefore, determinations of Cu speciation in sunlit surface waters may potentially lead to somewhat ambiguous results for free Cu^{2+} calculations, since some of the Cu(II) may in fact be Cu(I). Moffett and Zika (1988) have demonstrated that production of Cu(I) does occur during the day in the photic zone of open and coastal ocean waters. More recently, Buerge-Weirich and Sulzberger (2004) found that significant concentrations of Cu(I) (5-80% of TDCu) were formed in Scheldt estuary samples after light irradiation.

Although the effects of possible Cu(I) production should be limited in most estuaries and harbors to the uppermost part of the water column (probably less than 1-3 m in most cases), it seems prudent to suggest that Cu speciation sampling should be carried out at some standard depth (e.g., 3-5 m) at which sunlight should be sufficiently attenuated, to circumvent any possible ambiguity in free Cu^{2+} determinations.

4.13.2. Dredging effects and experimental design

As stated in project objectives 3 and 4, an important goal of the project was to determine how disturbances such as dredging could potentially affect Cu and Zn speciation and cycling in estuaries and harbors. These effects were primarily assessed by carrying out resuspension and photochemical experiments using authentic estuarine waters and sediments (see sections 4.8-4.11). In a secondary approach, we hoped to exploit the fact that the Cape Fear estuary was undergoing a straightening and dredging program approximately midway through the study period to examine how Cu and Zn speciation and cycling may be affected by these activities. In practice, we could not specifically plan our research cruises around the dredging schedule and maintain our regular sampling program at the same time, because of the considerable costs of vessel operation and analytical personnel. However, on the basis of our findings described in section 4, we can draw some conclusions on some potential impacts of dredging on metal speciation in section 5.7.

5. Conclusions

Based on the results of this research, we summarize the most important findings and offer specific recommendations regarding Cu and Zn speciation and cycling in harbor and estuarine environments.

5.1. Interlaboratory comparisons of total dissolved Cu and Zn and Cu and Zn speciation

Results of the interlaboratory comparison studies performed by CP-1156, CP-1157, and CP-1158 are described in section 4.12.1. Analyses for TDCu were statistically equivalent between groups, even in organic-rich samples such as those from the CFE. Copper speciation results using anodic stripping voltammetry and cathodic stripping voltammetry also agreed very well in the sense that they were coherently related to the analytical conditions (specifically the detection window as defined by the side reaction coefficient, α_{MAL}). In general, as the analytical detection window increases (α_{MAL} increases), the detected ligand concentrations generally become smaller. This is usually interpreted to mean that stronger ligands (with larger values of α_{MAL}) exist in smaller concentrations than do weaker ligands. The results of these studies are in very good agreement with the previous Cu speciation intercomparison described by Bruland et al. (2000).

Results from the TDZn and Zn speciation intercomparison are not as comprehensive as those for TDCu, reflecting the fact that two groups, CP-1156 and CP-1158, did not perform comprehensive Zn analyses as part of their research plans. In addition, there are far fewer speciation methods available for determining Zn speciation, in contrast to the various techniques used for Cu speciation.

5.2. Total dissolved Cu and Zn in the Cape Fear estuary

The EPA criterion continuous concentrations (CCC) in freshwater and saltwater, respectively, are 140 nM and 49 nM for TDCu and 1800 nM and 1200 nM for TDZn (EPA, 2002). Concentrations of TDCu (~3-25 nM) and TDZn (≤ 61 nM) determined in the CFE over the course of this work are well below the EPA CCC values for both freshwater and saltwater. The relatively low concentrations of TDCu and TDZn in the CFE probably reflect relatively small external inputs of these metals coupled with the rapid flushing rate (short residence time) of the estuary.

5.3. Dissolved Cu speciation in the Cape Fear estuary and implications for other estuarine systems

As described in Shank et al. (2004a) and summarized in section 4.2, the large excess of very strong Cu-complexing ligands ($\Sigma L_{i,Cu}$; 7 to >200 nM) relative to TDCu (~3-25 nM) maintains very low concentrations of free Cu^{2+} ($<10^{-13}$ M). The CFE system can therefore be considered to be very well “buffered” with respect to free Cu^{2+} ion. Experimental results from this study demonstrate that extracted humic substances account for virtually all the strong Cu-

complexing capacity in the CFE. Since humics are a relatively consistent fraction of the total DOC in the system, and DOC behaves conservatively in the CFE, we observed a strong correlation between $\Sigma L_{i,Cu}$ and DOC. This empirical relationship can be used to predict concentrations of $\Sigma L_{i,Cu}$ based on measurements of DOC. This is significant because the analysis of ligand concentrations is time-consuming and difficult, whereas DOC measurements are relatively simple. If this relationship can be established in other estuarine systems containing varying levels of DOC, TDCu, and $\Sigma L_{i,Cu}$, then DOC may become a useful proxy measurement for $\Sigma L_{i,Cu}$. Measurements of TDCu and DOC may therefore be used to relatively quickly estimate Cu speciation (including free Cu^{2+} concentrations) in estuarine or harbor systems.

5.4. Dissolved Zn speciation in the Cape Fear estuary and implications for other estuarine systems

Concentrations of TDZn ranged from non-detectable (ND) to 61 nM in the upper CFE (sta. 1) and ND to 61 nM in the lower estuary (sta. 2). Concentrations of $\Sigma L_{i,Zn}$ ranged from non-detectable to 102 nM throughout the estuary whereas TDZn concentrations ranged from non-detectable to 61 nM. In certain samples, $\Sigma L_{i,Zn}$ concentrations were less than TDZn, suggesting that TDZn can titrate out relatively strong ligands leaving the remaining Zn as either weakly complexed Zn or inorganic Zn. In contrast to the large extent of organic complexation of TDCu (>99%), only 25-84% of the TDZn occurs as relatively strong organic complexes in the CFE. This differing behavior is mainly a consequence of the much weaker nature (lower K') of the Zn- $\Sigma L_{i,Zn}$ complexes, as well as the comparatively lower concentrations of Zn ligands relative to TDZn in the CFE.

In contrast to the findings for Cu, extracted humic substances from the CFE did not significantly complex TDZn, indicating that humics are not a significant source of relatively strong Zn-complexing ligands in the CFE. Therefore, the ligands complexed to Zn and Cu are different and factors favoring strong Cu complexation in the CFE, and perhaps other estuarine and harbor systems, do not necessarily favor Zn complexation.

5.5. Benthic fluxes of Cu and Zn and Cu- and Zn-complexing ligands in the Cape Fear estuary and implications for other estuarine systems

Benthic fluxes of TDCu and TDZn were generally small and sporadic in the CFE system and did not contribute significantly (<5%) to observed standing stocks of these metals in the estuarine water column. This behavior presumably reflects relatively low concentrations of these metals in the sediments and porewaters. The relatively high flow rate of the system apparently keeps the sediment well-flushed, thereby precluding the accumulation of dissolved metals in the porewaters.

As discussed in detail in Shank et al. (2000c), benthic fluxes of strong Cu-complexing ligands were generally sporadic. A relatively small number of measurements showed significantly large outward fluxes of ligands. Since the effluxing ligands were not analytically distinguishable ($K'_{Cu-\Sigma L_i} \geq 10^{13}$) from those in the water column, it is possible that some of the ligands found in the CFE water column are derived from sediment porewaters. However, given

the large ligand concentrations found in the CFE water column and the short residence time of the system, benthic fluxes did not contribute significantly (<5%) to observed standing stocks. In estuarine systems possessing lower water column ligand concentrations and longer residence times (e.g., Chesapeake Bay), benthic fluxes of comparable magnitude could be a potentially significant source of strong Cu-complexing ligands.

Direct measurements of benthic fluxes of Zn-complexing ligands described in this study are the first ones reported. Similarly to fluxes of Cu ligands, Zn ligands fluxes are sporadic and contribute <5% to the total standing stock of ligands in the CFE water column. However, effluxing Zn ligands are analytically indistinguishable from those found in the water column; therefore, some of the water column ligands may be derived from sediment porewaters.

5.6. The role of photochemical processes in affecting the speciation and cycling of Cu in the Cape Fear estuary and implications for other estuarine systems

Experiments in which inorganic Cu^{2+} was added to natural CFE water and exposed to natural or simulated sunlight demonstrated that the magnitude of photobleaching or photochemical degradation of the colored dissolved organic matter (CDOM) was significantly reduced compared to experiments with no added Cu^{2+} . It is proposed that this photoprotection results from the formation of strong Cu complexes which act as a sink for superoxide radicals which would otherwise be available for photobleaching of CDOM. In the CFE, we calculate that up to 6% less photobleaching of CDOM occurs due to the ambient concentration of strongly complexed Cu. Thus, in the CFE and other estuarine and harbor systems, strongly complexed Cu alters the degree of photobleaching in waters exposed to sunlight.

Sunlight irradiation of natural CFE waters showed that 80-95% of the initial concentration of strong Cu-complexing ligands were photodegraded in full spectrum light and ~60% was degraded by photosynthetically available radiation (PAR) over the course of 4 days. The effectiveness of PAR in photodegrading ligands is significant because PAR can penetrate up to a few meters in even darkly colored estuarine waters. The overall importance of ligand photodegradation in natural waters depends upon many factors, including light intensity, depth of light penetration, and the physics of mixing processes. However, these observations suggest that photodegradation may be a significant sink for strong Cu-complexing ligands over the relatively long term.

Sunlight irradiation of complexed Cu produces significant concentrations of dissolved Cu(I), presumably by a ligand-to-metal charge transfer process. The Cu(I) produced is probably also strongly complexed also, although this can not be explicitly determined using current speciation methods. Transformations of Cu(I) and Cu(II) should show a strong diurnal cycle in estuarine surface waters, similar to what has been observed by Moffett and Zika (1988) in oceanic surface waters. Concentrations of dissolved Cu(I) in estuaries may therefore comprise a significant but variable fraction of total dissolved Cu, in agreement with recent findings by Buerge-Weirich and Sulzberger (2004) in the Scheldt estuary (Netherlands). The monitoring of Cu(I) in sunlight irradiations of unfiltered CFE waters and resuspended bottom sediments demonstrated that ambient suspended particles and sediments either have no impact on Cu(I)

production or cause a net uptake/oxidation of Cu(I). Thus resuspended sediments and ambient particles may be a net sink of Cu(I) in estuarine waters either through absorption or increased oxidation.

Resuspended natural CFE bottom sediments exposed to simulated sunlight did not appear to release or take up significant concentrations of TDCu, although some variable release was found after irradiation of unfiltered CFE water containing natural suspended particles but no bottom sediments. This suggests that the CFE suspended material, which probably contains more organic matter, may be more photoreactive than the bottom sediments. Resuspended San Diego Bay sediments, which contained much more Cu (2300 nmol Cu/g dry sediment) than the CFE sediments (3-20 nmol Cu/g dry sediment) showed a very large increase in total Cu concentration after sunlight irradiation. Similarly high release after photolysis was also observed in a relatively more contaminated CFE sediments adjacent to the Port of Wilmington (50 nmol/g dry sediment). Results of the photolysis experiments using the San Diego Bay and M61 sediments clearly indicate that photolytic release of Cu is only significant in sediments with relatively high Cu concentrations (such as San Diego Bay). In the CFE transect, percent organic carbon was highly correlated to the amount of Cu in the sediments. The association of Cu with organic components of sediments has been observed in other estuarine systems (Gerringa, 1990; Shaw et al., 1990; Turner et al., 2002; Shulkin and Bogdanova, 2003).

5.7. The effects of dredging on Cu and Zn cycling in the Cape Fear estuary

Only limited conclusions can be drawn regarding the specific effects of the large scale dredging and straightening project on Cu and Zn cycling in the Cape Fear estuary, since the design of the study did not permit the type of fine-scale sampling that is probably necessary to adequately assess dredging effects. The US Army Corps of Engineers (USACE) is currently sponsoring a multidisciplinary long-term monitoring project at UNCW to assess the effects of the dredging project on the Cape Fear ecosystem, although trace metal behavior is not explicitly being examined. Initial results from that work show, as expected, increased penetration of salt water in the upper portion of the system due to increased tidal volume (Hackney et al., 2003).

Our sediment results show that there are relatively low levels of total dissolved Cu and Zn in the CFE, and fluxes of these metals and their binding ligands do not significantly affect their standing stocks in the estuary. One of the major reasons for these observations is that the estuary is well-flushed, resulting in relatively short residence times (≤ 1 week) of the water. Since the dredging project is increasing the tidal volume of the system, this would serve to increase the flushing capability of the estuary, therefore leading to negligible effects upon Cu and Zn concentrations and speciation.

We found that resuspended sediments that are relatively high in Cu release metal when exposed to sunlight-exposed surface waters (see sections 4.10 and 5.6). However, we detected moderately elevated concentrations of sedimentary Cu only at sta. M61 adjacent to the Port of Wilmington; downstream sediments showed unremarkable concentrations of Cu. Therefore, for the bulk of the mainstem estuary, it is unlikely that resuspension of sediments will generate Cu contamination. We cannot specifically assess the effects of resuspension from dredging of

sediments upstream of M61; however, given the rapid flushing rate of the system, we would surmise that no significant long-term effects should be expected. We cannot assess potential Zn release from the sediments as that was not studied.

6. Literature Cited

- Aller R. C. (1982) The effects of macrobenthos on chemical properties of marine sediments and overlying waters, p. 53-102. In P.L. McCall and M.J.S.Tevesz [eds.], *Animal-Sediment Relations: The Biogenic Alteration of Sediments*. New York, Plenum.
- Anderson, D.M., Lively, J.S., and Vaccaro, R.F. (1984) Copper complexation during spring phytoplankton blooms in coastal waters. *J. Mar. Res.* 42:677-695.
- Anderson, B. S., Hunt, J.W. , Phillips, B.M., Fairey, R., Puckett, H.M., Stephenson, M., Taberski, K., Newman, J. and Tjeerdema, R.S. (2001) Influence of sample manipulation on contaminant flux and toxicity at the sediment-water interface. *Mar. Environ. Res.* 51:191-211.
- Apte, S.C., Gardner, M.J., and Ravenscroft, J.E. (1990) An investigation of copper complexation in the Severn estuary using differential pulse cathodic stripping voltammetry. *Mar. Chem.* 29:63-75.
- Avery, G.B., Willey, J.D., Kieber, R.J., Shank, G.C. and Whitehead, R.F., 2003. Flux and bioavailability of Cape Fear River and rainwater dissolved organic carbon to Long Bay, southeastern United States. *Global Biogeochem. Cycles* 17:1042-1047.
- Balzani, V. and Moggi, L. (1990) Photochemistry of coordination compounds: A glance at past, present, and future. *Coord. Chem. Rev.* 97:313-325.
- Behra, P. and Sigg, L. (1990) Evidence for redox cycling of iron in atmospheric water droplets. *Nature* 344:419-421.
- Berner, R.A. (1980) *Early Diagenesis: A Theoretical Approach*. Princeton, Princeton Univ. Press.
- Bertino, D.J. and Zepp, R.G. (1991) Effects of solar radiation on manganese oxide reactions with selected organic compounds. *Environ. Sci. Technol.* 25:1267-1273.
- Blough, N.V. and Zepp, R.G. (1995) Reactive oxygen species in natural waters, p. 280-333. In C.S. Foote, J.S. Valentine, A. Greenberg, and J.F. Liebman [eds.], *Active Oxygen in Chemistry*. New York, Chapman and Hall.
- Brand, L.E., Sunda, W.G., and Guillard, R.R.L. (1983) Limitation of marine phytoplankton reproductive rates by zinc, manganese, and iron. *Limnol. Oceanogr.* 28:1182-1195.
- Brand, L.E., Sunda, W.G., and Guillard, R.R.L. (1986) Reduction of marine phytoplankton reproduction rates by copper and cadmium. *J. Exp. Mar. Biol. Ecol.* 96:225-250.
- Bruland, K.W. (1980) Oceanographic distributions of cadmium, zinc, nickel, and copper in the North Pacific. *Earth Planet. Sci. Lett.* 47:176-198.
- Bruland, K.W. (1989) Oceanic zinc speciation: Complexation of zinc by natural organic ligands in the central North Pacific. *Limnol. Oceanogr.* 34:269-285.
- Bruland, K.W., Donat, J.R. and Hutchins, D.A. (1991) Interactive influences of bioactive trace metals on biological production in oceanic waters. *Limnol. Oceanogr.* 36:1555-1577.
- Bruland, K.W., Rue, E.L., Donat, J.R., Skrabal, S.A., and Moffett, J.W.(2000) Intercomparison of voltammetric techniques to determine the chemical speciation of dissolved copper in a coastal seawater sample. *Anal. Chim. Acta* 405:99-113.
- Buckley, J.M. and van den Berg, C.M.G. (1986) Copper complexation profiles in the Atlantic Ocean. A comparative study using electrochemical and ion exchange techniques. *Mar. Chem.* 19:281-296.

- Buerge-Weirich, D. and Sulzberger, B. (2004) Formation of Cu(I) in estuarine and marine waters: Application of a new solid-phase extraction method to measure Cu(I). *Environ. Sci. Technol.* 38:1843-1848.
- Burdige, D.J., and Homstead, J. (1994) Fluxes of dissolved organic carbon from Chesapeake Bay sediments. *Geochim. Cosmochim. Acta* 58:3407-3424.
- Byers, D. G. (1999). Zinc speciation and ligand fluxes in Chesapeake Bay and Elizabeth River pore waters. M.S. thesis, Old Dominion University, Norfolk, VA, 100 p.
- Cain, D.J. and Luoma, S.N. (1985) Copper and silver accumulation in transplanted and resident clams (*Macoma balthica*) in South San Francisco Bay. *Mar. Environ. Res.* 15:115-135.
- Campos, M.L.A.M. and van den Berg, C.M.G. (1994) Determination of copper complexation in sea water by cathodic stripping voltammetry and ligand competition with salicylaldoxime. *Anal. Chim. Acta* 284:481-496.
- Chadwick, D.B., Leather, J., Richter, K., Apitz, S., Lapota, D., Duckworth, D., Katz, C., Kirtay, V., Davidson, B., Patterson, A., Wang, P.F., Curtis, S., Key, J., Steinert, S., Rosen, G., Caballero, M., Groves, J., Koon, G., Valkirs, A., Meyers-Schulte, K., Stallard, M., Clawson, S., Streib-Montee, R., Sutton, D., Skinner, L., Germano, L. and Cheng, R. (1999) Sediment Quality Characterization Naval Station San Diego: Final Summary Report. SPAWAR Tech. Rep. 1777.
- Chadwick, D.B., Zirino, A., Rivera-Duarte, I., Katz, C.N. and Blake, A.C. (2004) Modeling the mass balance and fate of copper in San Diego Bay. *Limnol. Oceanogr.* 49:355-366.
- Coale, K.H. and Bruland, K.W. (1988) Copper complexation in the northeast Pacific. *Limnol. Oceanogr.* 33:1084-1101.
- Coale, K.H. and Bruland, K.W. (1990) Spatial and temporal variability in copper complexation in the North Pacific. *Deep-Sea Res.* 47:317-336.
- Cooper, D.C. and Morse, J.W. (1996) The chemistry of Offatts Bayou, Texas: A seasonally highly sulfidic basin. *Estuaries* 19:595-611.
- Cooper, D.C. and Morse, J.W. (1998) Biogeochemical controls on trace metal cycling in anoxic marine sediments. *Environ. Sci. Technol.* 32:327-330.
- Cooper, W.J., Zika, R.G., Petasne, R.G., and Plane, J.M.C. (1988) Photochemical formation of H₂O₂ in natural waters exposed to sunlight. *Environ. Sci. Technol.* 22:1156-1160.
- Cooper, W.J., Zika, R.G., Petasne, R.G., and Fischer, A.M. (1989) Sunlight-induced photochemistry of humic substances in natural waters: Major reactive species, p. 333-362. In I.H. Suffet and P. McCarthy [eds.], *Aquatic Humic Substances: Influence on Fate and Treatment of Pollutants*. Adv. Chem. 219, Am. Chem. Soc.
- Davies-Colley R.J., Nelson, P.O., and Williamson K.J. (1984) Copper and cadmium uptake by estuarine sedimentary phases. *Environ. Sci. Technol.* 18:491-499.
- Davis, J.A. (1984) Complexation of trace metals by adsorbed natural organic matter. *Geochim. Cosmochim. Acta* 48:679-691.
- Donat, J.R. and Bruland, K.W. (1990) A comparison of two voltammetric techniques for determining zinc speciation in northeast Pacific Ocean waters. *Mar. Chem.* 28:301-323.
- Donat, J.R., and Bruland, K.W. (1995) Trace elements in the oceans, p. 247-281. In E. Steinnes and B. Salbu [eds.], *Trace Elements in Natural Waters*. CRC Press.
- Donat, J.R., and van den Berg, C.M.G. (1992) A new cathodic stripping voltammetric technique for determining organic complexation of copper in seawater. *Mar. Chem.* 38:69-90.
- Donat, J.R., Lao, K.A., and Bruland, K.W. (1994) Speciation of dissolved copper and nickel in South San Francisco Bay: a multi-method approach. *Anal. Chim. Acta* 284:547-571.

- Elderfield, H., McCaffrey, R.J., Luedtke, N., Bender, M., and Truesdale, V.W. (1981a) Chemical diagenesis in Narragansett Bay sediments. *Am. J. Sci.* 281:1021-1055.
- Elderfield, H., Luedtke, N., McCaffrey, R.J., and Bender, M. (1981b) Benthic flux studies in Narragansett Bay. *Am. J. Sci.* 281:768-787.
- Ellwood, M. J. and van den Berg, C.M.G. (2000). Zinc speciation in the northeastern Atlantic Ocean. *Mar. Chem.* 68:295-306.
- Emerson, S., Jahnke, R., and Heggie, D. (1984) Sediment-water exchange in shallow water estuarine sediments. *J. Mar. Res.* 42:709-730.
- Faust, B.C. (1994) A review of the photochemical redox reactions of iron(III) species in atmospheric, oceanic, and surface waters: Influences on geochemical cycles and oxidant formation, p. 3-37. In G.R. Helz, R.G. Zepp, and D.G. Crosby [eds.], *Environmental Aspects of Surface and Aquatic Photochemistry*. Ann Arbor, Lewis Publ.
- Fischer, K., Dymond, J., Lyle, M., Soutar, A., and Rau, S. (1986) The benthic cycle of copper: Evidence from sediment trap experiments in the eastern tropical North Pacific Ocean. *Geochim. Cosmochim. Acta* 50:1535-1543.
- Fisher, N.S. and Wente, M. (1993) The release of trace metals by dying marine phytoplankton. *Deep-Sea Res.* 40:671-694.
- Flegal, A. R., and Sanudo-Wilhelmy, S.A. (1993). Comparable levels of trace metal contamination in two semi-enclosed embayments: San Diego Bay and South San Francisco Bay. *Environ. Sci. Technol.* 27:1934-1936.
- Gardner, M. (1999) Dissolved phase speciation of zinc in the Humber estuary. *Chemosphere* 38: 2117-2124.
- Gerringa, L.J.A. (1990) Aerobic degradation of organic matter and the mobility of Cu, Cd, Ni, Pb, Zn, Fe and Mn in marine sediment slurries. *Mar. Chem.* 29:355-374.
- Gerringa, L.J.A., Poortvliet, T.C.W. and Hummel, H. (1996) Comparison of chemical speciation of copper in the Oosterschelde and Westerschelde estuaries, The Netherlands. *Estuar. Coast. Shelf Sci.* 42:629-643.
- Gledhill, M., Nimmo, M., Hill, S.J., and Brown, M.T. (1997) The toxicity of copper(II) species to marine algae, with particular reference to macroalgae. *J. Phycol.* 33:2-11.
- Hackney, C.T., Posey, M., Leonard, L.L., Alphin, T., and Avery, G.B., Jr., 2003. Monitoring Effects of a Potential Increased Tidal Range in the Cape Fear River Ecosystem Due to Deepening Wilmington Harbor, North Carolina. Year 2: June 1, 2001-May 31, 2002. Report to the U.S. Army Corps of Engineers, Wilmington District, Contract DACW 54-02-0009, 226 p.
- Heggie, D., Klinkhammer, G.P., and Cullen, D. (1987) Manganese and copper fluxes from continental marine sediments. *Geochim. Cosmochim. Acta* 51:1059-1070.
- Henry, C. W., III (1996). Concentrations, Complexation and Speciation of Total Dissolved Zinc in the Chesapeake Bay. M.S. thesis, Old Dominion University, Norfolk, VA, 77 p.
- Hering J.G., Sunda W.G., Ferguson R.L., and Morel F.M.M. (1987) A field comparison of two methods for the determination of copper complexation: bacterial bioassay and fixed-potential amperometry. *Mar. Chem.* 20:299-312.
- Herzl, V. M. C., Millward, G.E., Wollast, R. and Achterberg, E.P. (2001). Species of dissolved Cu and Ni and their adsorption kinetics in turbid river water. *Estuar. Coastal Shelf Sci.* 56:43-52.
- Hong, H. and Kester, D.R. (1986) Redox state of iron in the offshore waters of Peru. *Limnol. Oceanogr.* 31:512-524.

- Huerta-Diaz, M.A. and Morse, J.W. (1992) Pyritization of trace metals in anoxic marine sediments. *Geochim. Cosmochim. Acta* 56: 2681-2702.
- ISO 10694 and BS7755 (1995) Determination of organic and total carbon after dry combustion. British Standard Methods of Tests for Soils for Civil Engineering Purposes: Part 3. Chemical Methods. British Standard Institution, London, Section 3.8.
- Jardim, W.F., and Pearson, H.W. (1984) A study of the copper-complexing compounds released by some species of cyanobacteria. *Water Res.* 18:985-989.
- Kerner, M. and Geisler, C.-D. (1995) Dynamics of Cu release during early aerobic degradation in aggregated seston from the Elbe estuary. *Mar. Chem.* 51:133-144.
- Kieber, R. J. and Helz, G.R. (1992). Indirect photoreduction of aqueous chromium(VI). *Environ. Sci. Technol.* 26: 307-312.
- Kieber, R.J., Li, A. and Seaton, P.J. (1999) Production of nitrite from the photodegradation of dissolved organic matter in natural waters. *Environ. Sci. Technol.* 33:993-998.
- Kieber, R.J., Skrabal, S.A., Smith, C., and Willey, J.D. (2004) Redox speciation of copper in rainwater: Temporal variability and atmospheric deposition. Accepted, *Environ. Sci. Technol.*
- Kozarac, Z., Plavsic, M., Cosovic, B., and Vilicic, D. (1989) Interaction of cadmium and copper with surface-active organic matter and complexing ligands released by marine phytoplankton. *Mar. Chem.* 26:313-330.
- Kozelka, P. B. and Bruland, K. W. (1998). Chemical speciation of dissolved Cu, Zn, Cd, Pb in Narragansett Bay, Rhode Island. *Mar. Chem.* 60:267-282.
- Kuwabara, J.S., Chang, C.C.Y., Khechfe, A., and Hunter, Y. (1996) Importance of dissolved sulfides and organic substances in controlling chemical speciation of heavy metals in San Francisco Bay, p. 157-172. In J.T. Hollibaugh [ed.], *San Francisco Bay: The Ecosystem*. Pacific Div. Am. Assoc. Adv. Sci.
- Landing, W.M. and Feely, R.A. (1981) The chemistry and vertical flux of particles in the northeastern Gulf of Alaska. *Deep-Sea Res.* 28A:19-37.
- Leal, M.F.C. and van den Berg, C.M.G. (1998) Evidence for strong copper(I) complexation by organic ligands in seawater. *Aqua. Geochem.* 4:49-75.
- Lee, B.-G. and Fisher, N.S. (1992) Degradation and elemental release rates from phytoplankton debris and their geochemical implications. *Limnol. Oceanogr.* 37:1345-1360.
- Lieseke, K.L. (2003) Benthic Fluxes and Speciation of Dissolved Zinc in the Cape Fear Estuary. M.S. thesis, University of North Carolina at Wilmington, Wilmington, NC, 45 p.
- Lion, L.W., Altmann, R.S., and Leckie, J.O. (1982) Trace-metal adsorption characteristics of estuarine particulate matter: evaluation of contribution of Fe/Mn oxide and organic coatings. *Environ. Sci. Technol.* 16:660-666.
- Luther, G.W., III, Rickard, D.T., Theberge, S., and Olroyd, A. (1996) Determination of metal (bi)sulfide stability constants of Mn^{2+} , Fe^{2+} , Co^{2+} , Ni^{2+} , Cu^{2+} , and Zn^{2+} by voltammetric methods. *Environ. Sci. Technol.* 30:671-679.
- MacGillivray, K.A. (2003) The Role of Benthic Macrofauna in Influencing Fluxes and Speciation of Dissolved Zinc and Copper in Estuarine Sediments. M.S. thesis, University of North Carolina at Wilmington, Wilmington, NC, 41 p.
- Mallin, M.A., Posey, M.H., Moser, M.L., Shank, G.C., McIver, M.R., Alphin, T.D., Ensign, S.H., and Merritt, J.F. (1998) Environmental Assessment of the Lower Cape Fear River System, 1997-1998. CMSR Rep. 98-02. Center for Marine Science Research, Univ. North Carolina at Wilmington, Wilmington, NC.

- Mallin, M. A., Posey, M.H., Lankford, T.E., McIver, M.R., CoVan, H.A., Alphin, T.D., Williams, M.S., and Merritt, J.F. (2003). Environmental assessment of the Lower Cape Fear River system, 2001-2002. Center for Marine Science, University of North Carolina at Wilmington, Wilmington, NC.
- McKnight, D.M., and Morel, F.M.M. (1979) Release of weak and strong copper-complexing agents by algae. *Limnol. Oceanogr.* 24:823-837.
- McKnight, D.M., and Morel, F.M.M. (1980) Copper complexation by siderophores from filamentous blue-green algae. *Limnol. Oceanogr.* 25:62-71.
- McKnight, D.M., Kimball, B.A., and Bencala, K.E. (1988) Iron photoreduction and oxidation in an acidic mountain stream. *Science* 240:637.
- Moffett, J.W. (1995) Temporal and spatial variability of copper complexation by strong chelators in the Sargasso Sea. *Deep-Sea Res. I* 42:1273-1295.
- Moffett, J.W. and Zika, R.G. (1987a) Solvent extraction of copper acetylacetonate in studies of copper(II) speciation in seawater. *Mar. Chem.* 21:301-313.
- Moffett, J.W. and Zika, R.G. (1987b) Reaction kinetics of hydrogen peroxide with copper and iron in seawater. *Environ. Sci. Technol.* 21:804-810.
- Moffett, J.W. and Zika, R.G. (1988) Measurement of copper(I) in surface waters of the subtropical Atlantic and Gulf of Mexico. *Geochim. Cosmochim. Acta* 52:1849-1857.
- Moffett, J.W. and Brand, L.E. (1996) Production of strong, extracellular Cu chelators by marine cyanobacteria in response to Cu stress. *Limnol. Oceanogr.* 41:388-395.
- Moffett, J.W., Zika, R.G., and Brand, L.E. (1990) Distribution and potential sources and sinks of copper chelators in the Sargasso Sea. *Deep-Sea Res.* 37:27-36.
- Moran, M.A., Sheldon Jr., W.M. and Sheldon, J.E., (1999) Biodegradation of riverine dissolved organic carbon in five estuaries of the southeastern United States. *Estuaries* 22: 55-64.
- Morel, F.M.M. and Hudson, R.J.M. (1985) The geobiological cycle of trace elements in aquatic systems: Redfield revisited, p. 2541-281. In W. Stumm [ed.], *Chemical Processes in Lakes*. New York, Wiley.
- Morse, J.W. and Arakaki, T. (1993) Adsorption and coprecipitation of divalent metals with mackinawite (FeS). *Geochim. Cosmochim. Acta* 57:3635-3640.
- Muller, F.L.L. and Kester, D.R. (1991) Voltammetric determination of the complexation parameters of zinc in marine and estuarine waters. *Mar. Chem.* 33:71-90.
- Murray, J.W. (1975) The interaction of metal ions at the manganese dioxide-solution interface. *Geochim. Cosmochim. Acta* 39:505-519.
- O'Connell, C. (1999) The Geochemistry of Silver and Zinc in the Cape Fear Estuary. M.S. thesis, University of North Carolina at Wilmington, Wilmington, NC, 40 p.
- Rivera-Duarte, I., and Flegal, A.R. (1997) Porewater gradients and diffusive benthic fluxes of Co, Ni, Cu, Zn, and Cd in San Francisco Bay. *Croat. Chem. Acta* 70:389-417.
- Robinson, M.G., and Brown, L.N. (1991) Copper complexation during a bloom of *Gymnodinium sanguineum* Hirasaka (Dinophyceae) measured by ASV. *Mar. Chem.* 33:105-118.
- Ruzic, I. (1982) Theoretical aspects of the direct titration of natural waters and its information yield for trace metal speciation. *Anal. Chim. Acta* 140:99-113.
- Seritti, A., Pellegrini, D., Morelli, E., Barghigiani, C., and Ferrara, R. (1986) Copper complexing capacity of phytoplanktonic cell exudates. *Mar. Chem.* 18:351-357.
- Shank, G.C. (2003) Processes Controlling the Geochemical Cycling of Strong Copper-Complexing Ligands in Estuarine and Marine Systems. Ph.D. dissertation, University of North Carolina at Chapel Hill, Chapel Hill, NC, 171 p.

- Shank, G.C., Skrabal, S.A., Whitehead, R.F., and Kieber, R.J. (2004a) Strong copper complexation in an organic-rich estuary: The importance of allochthonous dissolved organic matter. *Mar. Chem.* 88:21-39.
- Shank, G.C., Skrabal, S.A., Whitehead, R.F., Avery, G.B., and Kieber, R.J. (2004b) River discharge of strong Cu-complexing ligands to South Atlantic Bight waters. *Mar. Chem.* 88:41-51.
- Shank, G.C., Skrabal, S.A., Whitehead, R.F., and Kieber, R.J. (2004c) Fluxes of strong Cu-complexing ligands from sediments of an organic-rich estuary. *Estuar. Coast. Shelf Sci.* 60:349-358.
- Sharp, J.H., Benner, R., Carlson, C.A., Dow, R., and Fitzwater, S.E. (1993) Re-evaluation of high temperature combustion and chemical oxidation measurements of dissolved organic carbon in seawater. *Limnol. Oceanogr.* 38:1774-1782.
- Sharp, J.H., Benner, R., Bennett, L., Carlson, C.A., Fitzwater, S.E., Peltzer, E.T., and Tupas, L.M. (1995) Analyses of dissolved organic carbon in seawater: The JGOFS EqPac methods comparison. *Mar. Chem.* 48:91-108.
- Shaw, T.J., Gieskes, J.M., and Jahnke, R.A. (1990) Early diagenesis in differing depositional environments: the response of transition metals in pore water. *Geochim. Cosmochim. Acta* 54:1233-1246.
- Shea, D., and MacCrehan W.A. (1988). Role of biogenic thiols in the solubility of sulfide minerals. *Sci. Tot. Env.* 73:135-141.
- Shulkin, V. N. and Bogdanova, N.N. (2003). Mobilization of metals from riverine suspended matter in seawater. *Mar. Chem.* 83:157-167.
- Skrabal, S.A., Donat, J.R., and Burdige, D.J. (1997) Fluxes of copper-complexing ligands from estuarine sediments. *Limnol. Oceanogr.* 42:992-996.
- Skrabal, S.A., Donat, J.R., and Burdige, D.J. (2000) Pore water distributions of dissolved copper and copper-complexing ligands in estuarine sediments. *Geochim. Cosmochim. Acta* 64:1843-1857.
- Smith, M.L. (2003) Impact of Sediment Resuspension and Photochemistry on Dissolved Organic Carbon and Copper Speciation. M.S. thesis, University of North Carolina at Wilmington, Wilmington, NC, 56 p.
- Stumm, W. and Morgan, J.J. (1996) *Aquatic Chemistry*, 3rd Ed. New York, Wiley.
- Sturznegger, V.T. (1989) Hydrogen Peroxide in Surface Waters: Photochemical Production and Decay. Ph.D. thesis, Eidgenoessischen Technischen Hochschule, Zurich.
- Sulzberger, B., Suter, D., Siffert, C., Banwart, B., and Stumm, W. (1989) Dissolution of Fe(III) (hydr)oxides in natural waters: Laboratory assessment on the kinetics controlled by surface chemistry. *Mar. Chem.* 28:127-144.
- Sunda, W.G., and Guillard, R.R.L (1976) The relationship between cupric ion activity and the toxicity of copper to phytoplankton. *J. Mar. Res.* 34:511-529.
- Sunda, W.G. and Huntsman, S.A. (1983) Effect of competitive interactions between manganese and copper on cellular manganese and growth in estuarine and oceanic species of the diatom *Thalassiosira*. *Limnol. Oceanogr.* 28:924-934.
- Sunda W.G. and Hanson A.K. (1987) Measurement of free cupric ion concentration in seawater by a ligand competition technique involving copper sorption onto C18 Sep Pak cartridges. *Limnol. Oceanogr.* 32:537-551.
- Sunda, W.G. and Huntsman, S.A. (1988) Effect of sunlight on redox cycles of manganese in the southwestern Sargasso Sea. *Deep-Sea Res.* 35:1297-1317.

- Sunda, W.G. and Huntsman, S.A. (1991) The use of chemiluminescence and ligand competition with EDTA to measure copper concentration and speciation in seawater. *Mar. Chem.* 36:137-163.
- Sunda, W.G. and Huntsman, S.A. (1996) Antagonisms between cadmium and zinc toxicity and manganese limitation in a coastal diatom. *Limnol. Oceanogr.* 41:373-387.
- Sunda, W.G., Huntsman, S.A., and Harvey, G.R. (1983) Photoreduction of manganese oxides in seawater and its geochemical and biological implications. *Nature* 301:234-236.
- Sunda, W.G., Tester, P.A., and Huntsman, S.A. (1987) Effects of cupric and zinc ion activities on the survival and reproduction of marine copepods. *Mar. Biol.* 94:203-210.
- Sunda, W.G., Tester, P.A., and Huntsman, S.A. (1990) Toxicity of trace metals to *Acartia tonsa* in the Elizabeth River and southern Chesapeake Bay. *Estuarine Coastal Shelf Sci.* 30:207-221.
- Tang, D., Hung, C., Warnken, K.W. and Santschi, P.H. (2000). The distribution of biogenic thiols in surface waters of Galveston Bay. *Limnol. Oceanogr.* 45:1289-1297.
- Tang, D., Warnken, K.W. and Santschi, P.H. (2001) Organic complexation of copper in surface waters of Galveston Bay. *Limnol. Oceanogr.* 46:321-330.
- Tessier, A., Fortin, D., Belzile, N., DeVitre, R.R., and Leppard, G.G. (1996) Metal sorption to diagenetic iron and manganese oxyhydroxides and associated organic matter: Narrowing the gap between field and laboratory measurements. *Geochim. Cosmochim. Acta* 60: 387-404.
- Town, R.M. and Filella, M., 2000. Dispelling the myths: Is the existence of L1 and L2 ligands necessary to explain metal ion speciation in natural waters? *Limnol. Oceanogr.* 45:1341-1357.
- Turner, A., Martino, M. and Le Roux, S.M. (2002). Trace metal distribution coefficients in the Mersey Estuary, UK: Evidence for salting out of metal complexes. *Environ. Sci. Technol.* 36:4578-4584.
- van den Berg, C.M.G. (1982) Determination of copper complexation with natural organic ligands in seawater by equilibration with MnO_2 . II. Experimental procedures and application to surface seawater. *Mar. Chem.* 11:323-342.
- van den Berg, C.M.G. (1984) Determination of the complexing capacity and conditional stability constants of complexes of copper (II) with natural organic ligands in seawater by cathodic stripping voltammetry of copper-catechol complex ions. *Mar. Chem.* 15:1-18.
- van den Berg, C.M.G. (1985) Determination of the zinc complexing capacity in seawater by cathodic stripping voltammetry of zinc-APDC complex ions. *Mar. Chem.* 16:121-130.
- van den Berg, C.M.G. (1986) Determination of copper, cadmium, and lead in seawater by cathodic stripping voltammetry of complexes with 8-hydroxyquinoline. *J. Electroanal. Chem.* 215:111-121.
- van den Berg, C.M.G., and Dharmvanij, S. (1984) Organic complexation of zinc in estuarine interstitial and surface water samples. *Limnol. Oceanogr.* 29:1025-1036.
- van den Berg, C.M.G. and Donat, J.R. (1992) Determination and data evaluation of copper complexation by organic ligands in sea water using cathodic stripping voltammetry at varying detection windows. *Anal. Chim. Acta* 257: 281-291.
- van den Berg, C.M.G., Buckley, P.J.M., Huang, Z.Q., and Nimmo, M. (1986) An electrochemical study of the speciation of copper, zinc and iron in two estuaries in England. *Est. Shelf Sci.* 22:479-486.

- van den Berg, C.M.G., Merks, A.G.A., and Duursma, E.K. (1987) Organic complexation and its control of the dissolved concentrations of copper and zinc in the Scheldt estuary. *Est. Coast. Shelf Sci.* 24:785-797.
- van den Berg, C.M.G., Nimmo, M., Daly, P., and Turner, D.R. (1990) The effect of the detection window on the determination of organic copper speciation in estuarine waters. *Anal. Chim. Acta* 232:149-159.
- Velasquez, I. B., Jacinto, G. S. and Valera, F. S. (2002). The speciation of dissolved copper, cadmium and zinc in Manila Bay ,Philippines. *Mar. Poll. Bull.* 45:210-217.
- Voelker, B.M. and Kogut, M.B., 2001. Interpretation of metal speciation data in coastal waters: the effects of humic substances on copper binding as a test case. *Mar. Chem.* 74:303-318.
- Voelker, B.M., Sedlak, D.L. and Zafiriou, O.C. (2000) Chemistry of superoxide radical in seawater: Reactions with organic Cu complexes. *Environ. Sci. Technol.* 34:1036-1042.
- Waite, T.D. (1986) Photoredox chemistry of colloidal metal oxides, p. 426-445. In J.A. Davis and K.F. Hayes [eds.], *Geochemical Processes at Mineral Surfaces*. Washington, DC, Amer. Chem. Soc.
- Waite, T.D. (1990) Photo-redox processes at the mineral-water interface, p. 559-603. In M.F. Hochella, Jr. and A.F. White [eds.], *Mineral-Water Interface Geochemistry*. Washington, DC, Mineralogical Soc. Am.
- Waite, T.D. and Szymczak, R. (1993) Manganese dynamics in surface waters of the eastern Caribbean. *J. Geophys. Res.* 98:2361-2369.
- Waite, T.D., Wrigley, I.C., and Szymczak, R. (1988) Photoassisted dissolution of a colloidal manganese oxide in the presence of fulvic acid. *Environ. Sci. Technol.* 22:778.
- Warnken, K. W., Gill, G. A., Griffin, L. L. and Santshi, P. H. (2001) Sediment-water exchange of Mn, Fe, Ni, and Zn in Galveston Bay, Texas. *Mar. Chem.* 73:215-231.
- Wells, M.L., Kozelka, P.B. and Bruland, K.W. (1998) The complexation of 'dissolved' Cu, Zn, Cd and Pb by soluble colloidal organic matter in Narragansett Bay, RI. *Mar. Chem.* 62:203-217.
- Westerlund, S.F.G., Anderson, L.G., Hall, P.O.J., Iverfeldt, A., Rutgers van der Loeff, M.M., and Sundby, B. (1986) Benthic fluxes of cadmium, copper, nickel, zinc and lead in the coastal environment. *Geochim. Cosmochim. Acta* 50:1289-1296.
- Whitehead, R.F., de Mora, S.J., and Demers, S. (2000) Enhanced UV radiation—a new problem for the marine environment. In: S. de Mora, S. Demers, and M. Vernet (Eds.) *The Effects of UV Radiation in the Marine Environment*. Cambridge Environmental Chemistry Series 10. Cambridge University Press, Cambridge, p. 1-34.
- Xue, H. and Sigg, L. (1999) Comparison of the complexation of Cu and Cd by humic and fulvic acids and by ligands observed in lake waters. *Aqua. Geochem.* 5:313-335
- Young, L.B. and Harvey, H.H. (1992) The relative importance of manganese and iron oxides and organic matter in the sorption of trace metals by surficial lake sediments. *Geochim. Cosmochim. Acta* 56:1175-1186.
- Zafiriou, O.C. (1990) Chemistry of superoxide ion-radical (O_2^-) in seawater. I. pK_{asw}^* (HOO) and uncatalyzed dismutation kinetics studied by pulse radiolysis. *Mar. Chem.* 30:31-43.
- Zhou, X. and Wangersky, P.J. (1989) Production of copper-complexing ligands by the marine diatom *Phaeodactylum tricornutum* in a cage culture turbidostat. *Mar. Chem.* 26:239-259.

- Zuo, Y. and Hoigne, J. (1992) Formation of hydrogen peroxide and depletion of oxalic acid in atmospheric water by photolysis of iron(III)-oxalate complexes. *Environ. Sci. Technol.* 26:1014-1022.
- Zwolsman, J.J.G., Van Eck, B.T.M., and Van Der Weijden, C.H. (1997) Geochemistry of dissolved trace metals (cadmium, copper, zinc) in the Scheldt estuary, southwestern Netherlands: Impact of seasonal variability. *Geochim. Cosmochim. Acta* 61:1635-1652.

Appendix A: Supporting Data

Supporting data have been included in the main body of this report.

Appendix B: Technical Publications

B.1. Articles or papers published in peer-reviewed journals

- Shank, G.C., Skrabal, S.A., Whitehead, R.F., Avery, G.B., and Kieber, R.J. (2004) River discharge of strong Cu-complexing ligands to South Atlantic Bight waters. *Mar. Chem.* 88:41-51.
- Shank, G.C., Skrabal, S.A., Whitehead, R.F., and Kieber, R.J. (2004) Fluxes of strong Cu-complexing ligands from sediments of an organic-rich estuary. *Estuarine Coast. Shelf Sci.* 60:349-358.
- Shank, G.C., Skrabal, S.A., Whitehead, R.F., and Kieber, R.J. (2004) Strong copper complexation in an organic-rich estuary: The importance of allochthonous dissolved organic matter. *Mar. Chem.* 88:21-39.

B.2. Technical reports

- Skrabal, S.A., Kieber, R.J., and Cooper, W.J. (2001) Speciation, Fluxes, and Cycling of Dissolved Copper and Zinc in Estuaries: The Roles of Sediment-Water Exchange and Photochemical Effects (CP-1157). Annual Report to Strategic Environmental Research and Development Program, Arlington, VA, 12 p.
- Skrabal, S.A., Kieber, R.J., and Cooper, W.J. (2002) Speciation, Fluxes, and Cycling of Dissolved Copper and Zinc in Estuaries: The Roles of Sediment-Water Exchange and Photochemical Effects (CP-1157). Annual Report to Strategic Environmental Research and Development Program, Arlington, VA., 11 p.

B.3. Conference/symposium proceedings and/or papers scientifically recognized and referenced (other than abstracts)

- Lieseke, K.L. (2003) Benthic Fluxes and Speciation of Dissolved Zinc in the Cape Fear Estuary. M.S. thesis, University of North Carolina at Wilmington, Wilmington, NC, 45 p.
- MacGillivray, K.A. (2003) The Role of Benthic Macrofauna in Influencing Fluxes and Speciation of Dissolved Zinc and Copper in Estuarine Sediments. M.S. thesis, University of North Carolina at Wilmington, Wilmington, NC, 41 p.
- Shank, G.C. (2003) Processes Controlling the Geochemical Cycling of Strong Copper-Complexing Ligands in Estuarine and Marine Systems. Ph.D. dissertation, University of North Carolina at Chapel Hill, Chapel Hill, NC, 171 p.
- Skrabal, S.A. (2002) Determination of Cu ligands by voltammetry, p. 59-62. In: *Copper Chemistry, Toxicity, and Bioavailability and Its Relationship to Regulation in the Marine Environment*. Office of Naval Research Second Workshop Report (A. Zirino and P.F. Seligman, eds.). Technical Document 3140, SPAWAR Systems Center, San Diego, CA.

Smith, M.L. (2003) Impact of Sediment Resuspension and Photochemistry on Dissolved Organic Carbon and Copper Speciation. M.S. thesis, University of North Carolina at Wilmington, Wilmington, NC, 56 p.

B.4. Published technical abstracts

Skrabal, S.A. (2000) Copper ligands by voltammetry. 2nd Office of Naval Research Workshop on Chemistry, Toxicity, and Bioavailability of Copper and its Relationship to Regulation in the Marine Environment. Annapolis, MD.

Skrabal, S.A., Kieber, R.J., and Willey, J.D. (2000) Redox-active trace metals in rainwater and surface waters: Speciation and photochemical effects. American Chemical Society Pacificchem 2000 meeting, Honolulu, HI.

Skrabal, S.A., Kieber, R.J., and Willey, J.D. (2001) Redox-active trace metals in rainwater and surface waters: Speciation and photochemical effects. 5th Annual UNCW Symposium on Chemistry and Biochemistry, Wilmington, NC.

Whitehead, R.F., Shank, G.C., Skrabal, S.A., Kieber, R.J., Cooper, W.J. (2001) Photochemical interactions with copper-complexing DOM in the Cape Fear Estuary, NC. American Society of Limnology and Oceanography Aquatic Sciences Meeting, February 2001, Albuquerque, NM

Skrabal, S.A. (2001) Fluxes and cycling of dissolved copper and zinc in estuaries. Partners in Environmental Technology Technical Symposium and Workshop, Washington, DC).

Whitehead, R.F., Skrabal, S.A., Shank, G.C., and Kieber, R.J. (2002) The influence of copper speciation on the photobleaching of CDOM in the Cape Fear Estuary. 6th Annual UNCW Symposium on Chemistry and Biochemistry, Wilmington, NC.

Smith, M.L., Kieber, R.J., Skrabal, S.A., and Whitehead, R.F. (2002) Photochemically initiated changes in copper speciation during sediment resuspension events. 6th Annual UNCW Symposium on Chemistry and Biochemistry, Wilmington, NC.

Shank, G.C., Skrabal, S.A., Whitehead, R.F., and Kieber, R.J. (2002) Mechanisms controlling strong copper-complexing ligands in the Cape Fear estuary, North Carolina. American Geophysical Union Ocean Sciences Meeting, Honolulu, HI.

Shank, G.C., Skrabal, S.A., Whitehead, R.F., and Kieber, R.J. (2002) Mechanisms controlling dissolved copper speciation in the Cape Fear estuary, North Carolina, USA. Seventh International Estuarine Biogeochemistry Symposium, Grimstad, Norway.

Smith, M.L., Kieber, R.J., Skrabal, S.A., Whitehead, R.F., and Shank, G.C. (2002) Photochemically initiated changes in copper speciation during sediment resuspension. 54th Southeast Regional Meeting of the ACS, Charleston, SC.

Skrabal, S.A., Whitehead, R.F., Kieber, R.J., Cooper, W.J., Shank, G.C., Lieseke, K., MacGillivray, K., and Smith, M. (2002) Speciation, fluxes and cycling of dissolved copper and zinc in estuaries: The roles of sediment-water exchange and photochemical effects. Partners in Environmental Technology Technical Symposium and Workshop, Washington, DC.

Skrabal, S.A., Shank, G.C., Whitehead, R.F., Kieber, R.J., MacGillivray, K.A., and Lieseke, K.L. (2003) Copper and zinc complexing ligands in estuarine waters: The role of sediment-water exchange. 225th American Chemical Society National Meeting, New Orleans, LA.

- Whitehead, R.F., Smith, M.L., Kieber, R.J., Skrabal, S.A., and Shank, G.C. (2003)
Photochemically initiated changes of DOC and copper speciation during sediment
resuspension events. 225th American Chemical Society National Meeting, New Orleans,
LA.
- Skrabal, S.A., Kieber, R.J., Whitehead, R.F., Shank, G.C., Smith, M.L. and Lieseke, K.L. (2003)
Speciation, fluxes and cycling of dissolved copper and zinc in estuaries: The roles of
sediment-water exchange and photochemical effects. Partners in Environmental
Technology Technical Symposium and Workshop, Washington, DC.
- Skrabal, S.A., Smith, M.L., Kieber, R.J., Shank, G.C., and Whitehead, R.F. (2004) Dissolved
copper in organic-rich estuarine waters: Interactions of light and suspended sediments in
altering speciation. American Society of Limnology and Oceanography/Oceanography
Society Ocean Research Conference, Honolulu, HI.

B.5. Published text books or book chapter

None

Appendix C: Other Technical Material

C.1. Scientific/technical honors received

1. Strategic Environmental and Research Development Program compliance project of the year award (2003). *Speciation, Fluxes, and Cycling of Dissolved Copper and Zinc in Estuaries: The Roles of Sediment-Water Exchange and Photochemical Effects*
2. Project described in “Success Stories” in SERDP/ESTCP Information Bulletin, Spring 2004, Number 20.